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MANUAL FOR THE PHYSIOLOGICAL LABORATORY.

BY

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PREFACE TO THE FOURTH EDITION.

THE present edition is considerably altered. The Introduction has been enlarged, the Histology revised, and the Chemical Physiology considerably added to and improved.

The Physiology Proper has also undergone some modification. In each division of the book much new matter has been introduced.

The object of the authors in this edition, as in the former ones, has been to make the book of real practical value to the student, and not to burden him with descriptions of methods which are not within his scope. The alterations have been suggested by the experience of many years' teaching, and we think that they will be found to add to the usefulness of the book.

To our colleagues, Dr. Lewis Jones, Dr. Tooth, and Dr. Shore, we would tender our most hearty thanks for the help which they have at all times so readily afforded to us ; and more especially for the assistance which they have rendered in re-casting

Parts II. and III. Dr. Jones has further increased our obligation to him by the care and time which he has expended in reading the proof-sheets of the present edition, thereby correcting many errors and inaccuracies into which we should otherwise have fallen.

To Mr. John Murray and to Messrs. Churchill, as well as to several scientific instrument-makers who have allowed us the use of their illustrations and plates, we also beg to tender our best thanks.

CORRIGENDA.

Page 24, *line* 1, read 'Paraffin.'
„ 163, „ 31, *for* 'sulphindigotate of carmine' *read* 'sulphindigotate of soda or indigo carmine.'

PART I.

PRACTICAL HISTOLOGY.

CHAPTER I.

THE MICROSCOPE AND MICROSCOPICAL METHODS.

THE subject of practical histology may be conveniently introduced by an account, firstly, of the microscope and its accessory apparatus, and, secondly, of the methods of preparing tissues in order that their structure may be adequately studied with the aid of that instrument.

The present chapter, therefore, will be devoted to a description, as brief as possible, of these two parts of the subject in turn.

THE MICROSCOPE AND ITS ACCESSORY APPARATUS.

The form of microscope which we select for description is the one generally known as the **Continental Model** (Fig. 1). We choose it because of its simplicity. Although originally it was manufactured by foreign makers only, and differed in plan from most of the English microscopes, at the present time instruments very similar in construction, although differing somewhat in detail, are made by nearly all the best English makers.

The model is often called the **Hartnack Model**, as it was chiefly by means of the instruments of that make that the small continental microscope became popular in this country.

In this model the stand or body of the instrument consists of two parts. Of a base of solid brass, into which is fixed vertically a substantial brass pillar about two inches high, and to which is attached by a hinge the second portion of the stand. This latter is made up of a hollow cylinder of brass, carrying below at right angles the stage of the microscope, and above, by a projecting arm, a long brass collar, in which the tube of the

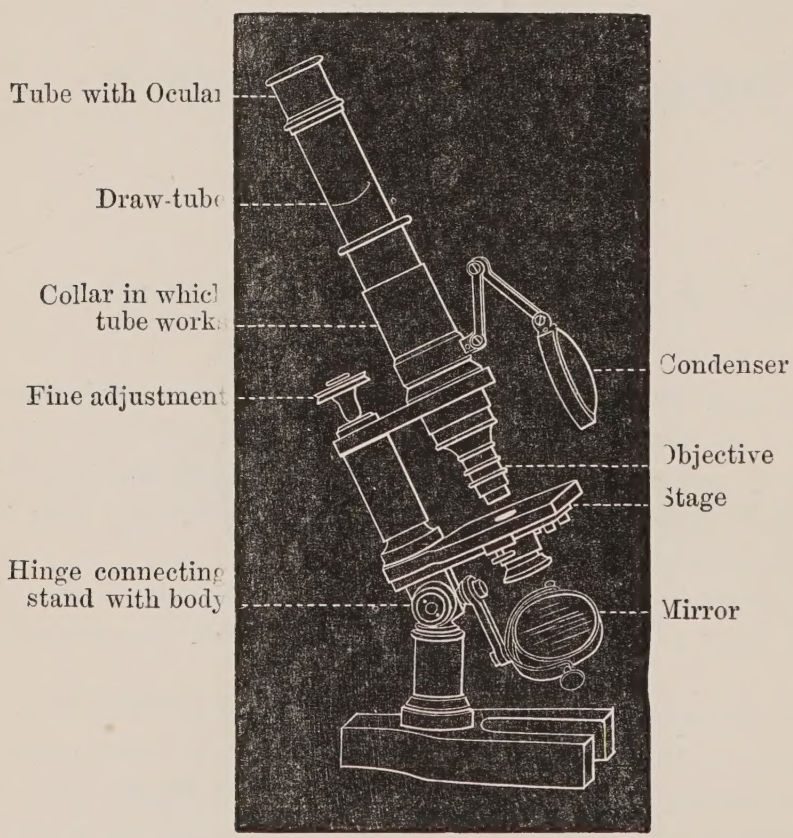

Fig. 1.—Continental Model Microscope.

microscope slides. At the top of the hollow cylinder is a screw with a milled head which works the **fine adjustment** ; when the screw is turned the tube of the microscope is moved very slightly to or from the stage. In the simpler forms the **coarse adjustment**, or the freer movement of the microscope tube up and down, is effected by the hand, whilst in the more expensive models it is performed by a rack and pinion. The **stage** of the microscope, upon which the object-glass is placed, is a flat plate

of brass blackened upon its upper surface, or of glass cemented on to a dark background, and perforated by a central aperture. Through the aperture light is reflected from a **mirror**; the amount of light admitted to the lens is regulated by a small cylinder which fits into the central aperture; this, when withdrawn from below, will receive the **diaphragms**; these are perforated by apertures of different sizes; the smallest is a mere pinhole, and is for use with the strongest magnifying power. One of the diaphragms should be inserted into the cylinder, and replaced in the centre of the stage. When in position it is exactly flush with the upper surface. In some microscopes this form of diaphragm is replaced by a blackened disc of metal perforated near its circumference by holes of various sizes, and revolving round its centre. At the back of the stage is a pair of brass **clips** for holding the slides in position when the microscope is tilted. In some instruments the movements of the stage in different directions are effected by screws; but in this the stage is fixed, and the finger and thumb suffice to move the slide upon it.

Beneath the stage is a movable mirror with two faces, one concave, the other plane; the concave mirror is the more commonly used, as it condenses the light upon the object and thus affords a better illumination. The **tube** of the microscope consists of a hollow brass cylinder from five to eight and a half inches in length. It contains a second or **draw**-tube, which can, if necessary, be drawn out, in order to increase the magnifying power. The upper extremity of the tube receives the **ocular** or eye-piece: the oculars vary in magnifying power. It is better not to change the oculars, but always to work with one of moderate power.

Into the lower end of the tube the **objectives, powers,** or **lenses** should be screwed. These also vary in magnifying power.

In using the microscope, put on a low power first; then adjust the mirror in such a way as to get a full illumination of **the field,** so that the eye applied to the ocular sees a circle of light of equal intensity in all its parts. Never use the direct rays of the sun. Next put the object to be examined

upon the stage in such a way as to be over the centre of the aperture: the diaphragm with largest aperture is to be used. Bring the lens to within half an inch of the slide, and carefully employ the fine adjustment until the object is distinctly seen; then alter the focus so as to observe it at various depths. After examining with the lower power, remove this and replace it by higher powers, at the same time changing the diaphragm.

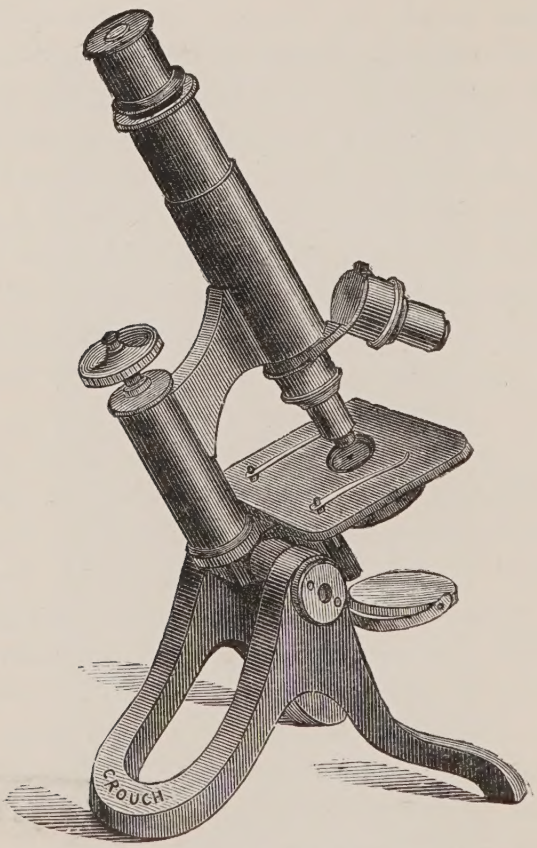

Fig. 2.—English Model of Students' Microscope: the nose-piece carries two objectives.

for a smaller one: bring the lens down until it almost touches the object, and then focus upward by means of the fine adjustment.

Students are apt to leave a portion of the lens attached to the tube when they change the powers; or perhaps the field may be obscured by drops of glycerine, Canada balsam, etc., adhering to the surface of the lens. Attention may be directed

to these points when it is found that the object cannot be focussed clearly. A drop of methylated spirit upon a soft handkerchief is sufficient to remove Canada balsam from lenses. A **condenser**, or convex lens fitted into a jointed lever which slides up and down the tube of the microscope, is provided ; it is employed for condensing light upon opaque specimens ; it may, however, be removed, since it is scarcely ever needed in modern histology, as the majority of specimens are prepared for examination by reflected light.

It will be seen by an examination of Fig. 2, which represents a student's microscope of English make, that it differs but little from that which we have described. The body or stand is, however, a tripod arrangement, which is the form at present in vogue in this country.

Before giving a brief description of the optical plan of a compound microscope, into the construction of which many lenses enter, we may mention, in passing, **the simple or dissecting microscope**, which consists essentially in a stage illuminated from below by a movable mirror. Above the stage is an arm which can be raised or lowered by a rack and pinion. The arm carries a lens similar to that employed by watchmakers. The dissecting microscope is serviceable in certain cases where it is necessary to tease or otherwise manipulate very delicate tissues. It is chiefly, however, of use to biologists.

The Mode in which the Compound Microscope Magnifies.—The rays of light (Fig. 3) which proceed from the object under examination are focussed behind the objective at a point in the lumen of the tube of the microscope the position of which is dependent upon the strength of the magnifying power employed. Thus the higher the objective, the nearer is the object focussed to the lower end of the tube ; and the less the magnifying power of the objective, the higher in the tube is the focus. After the various rays have been brought to a focus they cross each other and become divergent, until they are a second time gathered up by the front lens of the ocular, which focusses them within the eye-piece at the level of the diaphragm. An enlarged but inverted image of the object in front of the objective is thus formed, which is a second time magnified by the lens of the

ocular nearest to the eye. The magnifying power of the microscope is increased within certain limits by increasing the distance between the objective and the eye-piece, and it is for this reason that most of the modern instruments are provided with a 'draw-tube.' In using the draw-tube, however, as well as in viewing objects with highly magnifying eye-pieces, it must be borne in mind that it is only the image formed by the objective which is enlarged, and not the object itself, and with such increased magnification any defect or distortion on the part of the objective will be further amplified, and it is therefore best only to employ eye-pieces of moderate strength.

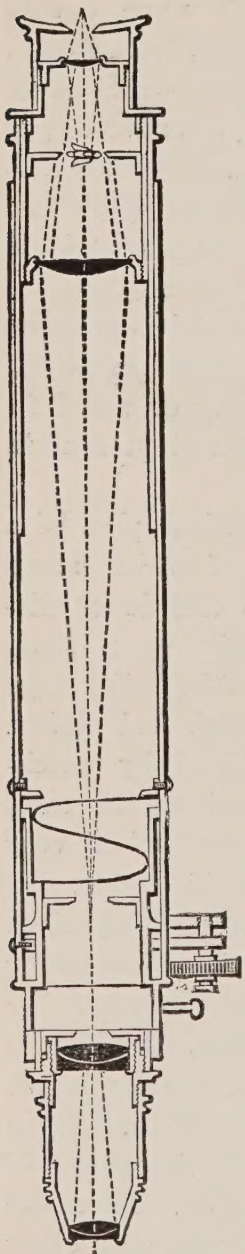

Fig. 3.—Diagram of the Method in which a Compound Microscope magnifies.

Lenses or Objectives.—In Fig. 3, the objective, although one of low magnifying power, is seen to be made up of combinations of lenses instead of single plano-convex glasses. This is the plan always adopted nowadays with the compound microscope. Objectives of high magnifying power are indeed always made up of more than two such combinations. The explanation is not far to seek.

Single plano-convex lenses are subject to two great defects, of which the first is **spherical aberration.** This results from the fact that rays of light which pass through the circumference of the lens are brought to a focus at a point nearer to the lens than the rays which pass through the centre. The second defect is that of **chromatic aberration,** consequent upon the complex nature of white light. The rays in passing through the lens are split up into their component parts, and thus the colours of the spectrum are obtained in the same way as when light passes through a prism. Each colour of the spectrum is focussed at a different point, and the

image is therefore surrounded by a coloured fringe. It has been found, however, that by a skilful combination of different kinds of glass, objectives can be manufactured in which the spherical and chromatic aberrations are practically done away with. Objectives, therefore, are usually composed of combinations of lenses, varying in number from two to eight, such as we have above-mentioned, and they are composed of flint and crown glass ; the flint-glass lenses being concave, and ground so as to fit the opposed convex surfaces of each crown-glass lens. In the better class of objectives the lenses farthest away from the object are ground in such a manner as not only

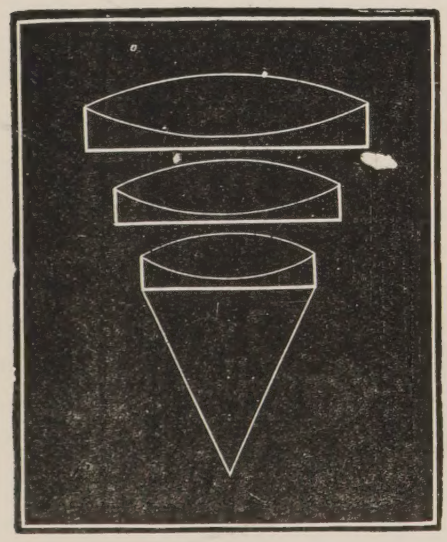

Fig. 4.—Old Form of Combination of Lenses.

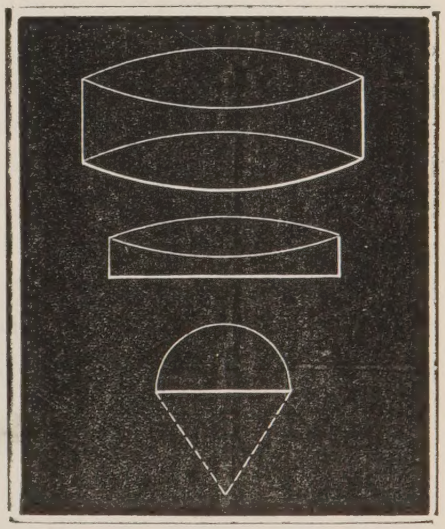

Fig. 5.—New Form of Combination of Lenses.

to correct their own spherical aberration, but also that of the front lens. By this means an objective is obtained in which the front lens consists of a single glass, and not, as in the older forms, of an achromatic combination of convex and concave glasses, which only allowed of a small angular aperture.

By the angular aperture of a lens is meant the number of rays of light which it is capable of transmitting. Such aperture is measured by observing, with a special instrument, the angle subtended from the centre of the objective by the most oblique ray of light transmitted on either side. For ordinary purposes an objective whose focal length is $\frac{1}{4}$ inch should not

possess a greater angular aperture than 100° ; whilst $\frac{1}{8}$-inch should not exceed 130°, for beyond this point the larger amount of light entering the objective is more than counterbalanced by the increased proximity of the lens to the object, and the diminished amount of penetrating power which such a glass possesses. This statement does not hold true for immersion lenses, in which the angular aperture can be increased to a very great extent without detriment to the utility of the objective.

Immersion Lenses.—The development of modern histology, especially in the direction of the recognition and cultivation of the exceedingly minute forms of vegetable life known as micro-organisms, has led to a corresponding demand for higher microscopic magnifying powers. This demand was at first met by the manufacture of the more powerful objectives which have already been described, in which an interval of air intervenes between the front of the lens and the surface of the glass covering the preparation. It was soon found, however, that such higher objectives focussed inconveniently near the cover-glass, whilst at the same time they had a very limited field, and did not admit of the passage of sufficient rays of light to yield a good illumination. A new form of objective has therefore been constructed of such a character that it focusses an object when the interval between the front lens and the cover-glass is occupied by some fluid which reduces to a minimum differences in the index of refraction. Such objectives are known as 'immersion' lenses, to distinguish them from the old form of 'dry' lens. Water, glycerine, and oils have been successively employed in connection with these lenses ; but cedar-oil condensed until it has a somewhat higher specific gravity than usual, is now most commonly used for the purpose, because its index of refraction is found to be very nearly equivalent to that of crown glass, and therefore nearly all the rays of light which are focussed upon the object pass through the objective into the body of the microscope.

To use an immersion lens the mounted specimen must be sealed with marine glue, gold-size, or other cement which is not soluble in cedar-oil. A small drop of the clear oil is then lightly placed, by means of a clean glass rod, upon that portion

of the cover-glass which is immediately over the part of the preparation to be examined. The lens is afterwards brought down carefully until its surface is in actual contact with the oil, and it is then focussed by means of the fine adjustment. The immersion lenses employed in histological research are usually without correction for the thickness of the cover-glass, as they are more readily employed than when a different correction of the lens has to be made for each thickness of cover-glass employed. Before using the immersion lens it will usually be found expedient to find the particular spot in the preparation with an ordinary high power, and if the specimen is permanent the part required should be marked out for future reference by drawing round it upon the cover-glass a small ring of cement or ink.

In the majority of cases a special form of microscope will be required, as it will be necessary, in order to obtain a sufficiently powerful illumination, to attach an achromatic condenser beneath the stage. After using the immersion lens it should be at once unscrewed, its front surface should be gently wiped with a soft handkerchief or piece of chamois leather kept for the purpose, and it should then be replaced in its box.

Oculars or eye-pieces are of two great types. The Huyghenian is in common use, and consists of two plano-convex lenses, separated by a distance equal to half the sum of their combined focal length, with a diaphragm placed between them. The Kellner, or orthoscopic eye-piece, is chiefly employed in micro-photography, and is composed of an upper achromatic lens, and of a lower bi-convex lens placed in the focus of the upper or eye lens. It has no intervening diaphragm, and therefore has nearly double the field of view possessed by the Huyghenian eye-pieces.

Apochromatic Objectives.—Zeiss has recently introduced a new series of lenses, which he has termed Apochromatic. The particular advantages claimed for them are that in consequence of an improved method of correction, and the use of new kinds of glass, the spherical aberration is entirely abolished. The lenses can be employed with much higher eye-pieces than has hitherto been the case ; and the natural colour of objects, even

in the more delicate tints, are reproduced unaltered. To obtain the best results with these objectives, they should be employed with the compensating eye-pieces manufactured by the same firm ; when this is done an image is obtained which is uniformly free from colour throughout the whole field of view. Drysdale, after carefully testing this combination of ocular and objective, has come to the conclusion that results may be obtained with them which are equal but not superior to those yielded by the immersion lenses of the best English makers.

Light.—The best light for microscopic work is that afforded by the sun, when its rays are not too powerful. A fine sunny spring day affords what may be called a typically favourable light. The light should be considered best when reflected from white clouds in a blue sky. In England, however, only too frequently artificial light has to be made use of. Various forms of gas and oil lamps have been suggested. A drawing of a convenient form is given. As a rule we employ gas argand burners, with blue glass funnels. The light is generally transmitted to the object by a slightly concave mirror through a diaphragm. The illumination of objects when opaque is done by means of condensers of various kinds, which focus the light upon the object from above, or condense it laterally by parabolic side-reflectors. Opaque objects, however, as before mentioned, seldom need to be examined in histological work.

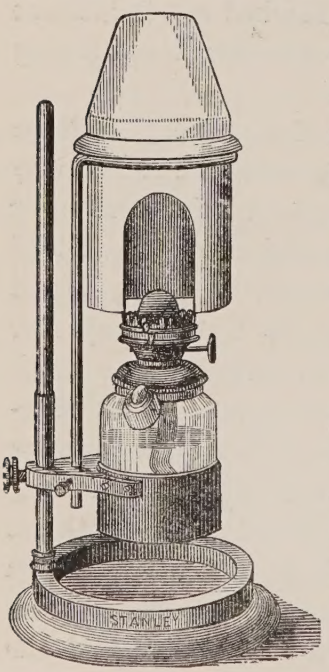

Fig. 6.—Microscope Lamp.

In using immersion objectives, the ordinary mirror is supplemented by some form of achromatic condenser, of which perhaps the illuminating apparatus designed by Abbe may be considered as the best. It consists essentially of two or more lenses, so combined as to transmit a large pencil of achromatic light, which is reflected either from a concave or plane mirror. The amount of light entering the instrument may be regulated by

introducing diaphragms ; whilst the direction of the rays can be varied at will by turning a milled head. The top lens, when the apparatus is in position, fits accurately into the central aperture in the stage of the microscope, and is flush with its upper surface.

Of Drawing Microscopical Objects.—If the student is a good draughtsman, practice is the only thing required before he can make good drawings of microscopic tissues. In others practice will very possibly bring out latent talents. Some, however, whatever their perseverance, will need aid ; and this is given them

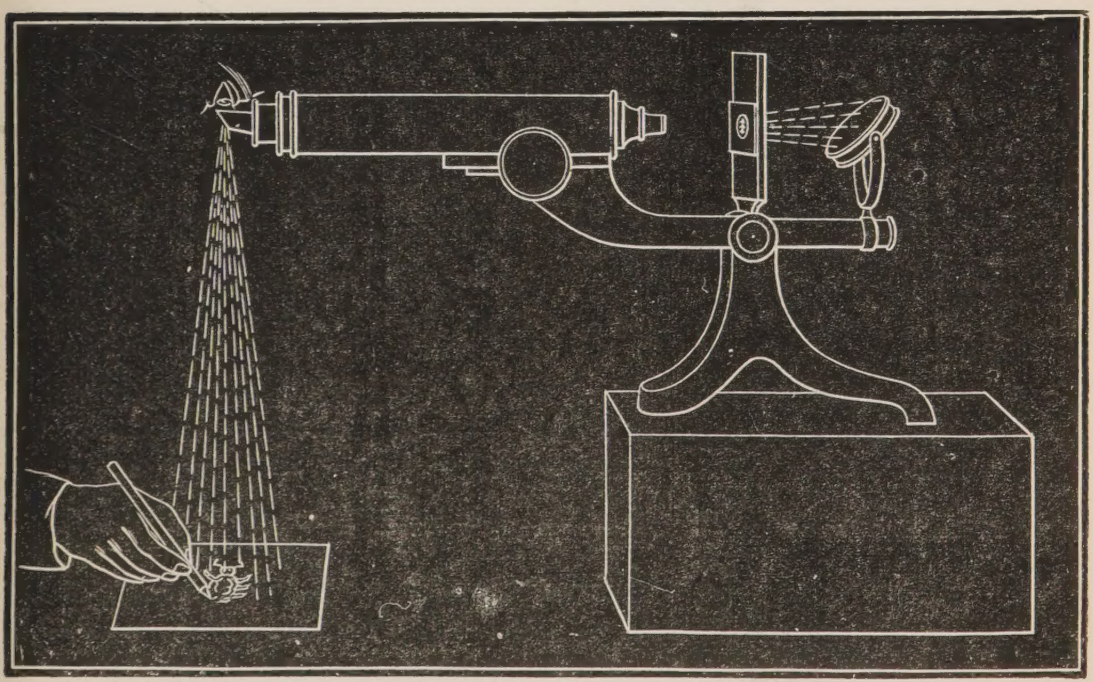

Fig. 7.—Diagram of the Position of the Microscope, etc., when using the Camera Lucida.

by an instrument called the **camera lucida**. This consists of a prism arranged in such a manner that, when attached to the microscope tube by a holder, and placed exactly over the eye-piece, the image of the object is thrown upon some part of the table where a sheet of white paper may be laid, and the outline of the object can then be traced upon it. An instrument constructed for a similar purpose is Beale's neutral tint reflector,

which is thus used: The cap of the eye-piece is removed, and the reflector is applied in place of it. The microscope should then be inclined to a horizontal position, and at ten inches from the table, and the paper is placed exactly underneath the reflector. After the object is focussed and properly illuminated, the eye should be brought *close to* and *exactly over* the reflector, and the image will then appear to be thrown upon the paper, and may readily be traced.

Directions for Choosing a Microscope.—Great care is necessary in the choice of a microsope, and the student is recommended not to buy one without asking the advice of some one who is well acquainted with modern instruments. The supply of different kinds is now so extensive, that a description of what should be chosen is difficult and might be invidious. We may, however, tell him roughly *what not to get.* Let him *not* buy one of those large constructions of brass which are so often strongly recommended by dealers, or one which has a complicated arrangement of screws and buttons to move the object-glass. This would be simply paying money for useless material. Binocular microscopes cannot be recommended, nor those in which the fine adjustment tilts the draw tube forward. Second-hand instruments, except modern instruments of well-known makers, are to be avoided, as are also old instruments, of whatever kind. The following points of advice may be of some use:

The stand must be small, and at the same time firm; the fine adjustment delicate and steadfast. The oculars ought to be clear and achromatic, free from flaws, scratches, and spherical aberration.

The lenses should also be free from any such faults, and should exhibit a flat field, the whole of the field being in focus at once; should have a fair power of penetration—*i.e.*, should be capable of showing the parts beyond the exact focus; and should also possess what is technically known as resolving power—*i.e.*, should be able to focus clearly a number of fine lines, close together, in an object.

Every part should be carefully tested, and if found defective should be returned to the maker after a fair trial, even if paid

for ; and, indeed, the best makers are anxious that no imperfect lenses bearing their names should be in current use.

Of course the question of *price* is a very important one—how much money is to be expended upon the purchase of an instrument ? It is seldom necessary to spend more, to begin with, and not expedient as a rule to spend less, than *five guineas*. For this money the student obtains a good stand, two eye-pieces, and two objectives, $\frac{2}{3}$-inch and $\frac{1}{5}$ or $\frac{1}{6}$, in a stout case ; in fact, all that he will be likely to require for a considerable time.

The accompanying figure of a Student's Microscope shows such an instrument. It may be obtained from one of the following makers :

BECK, 68, Cornhill ;
CROUCH, 66, Barbican ;
SWIFT, 81, Tottenham Court Road ;
PARKES, 5, St. Mary's Row, Birmingham ; or
STANLEY, London Bridge.

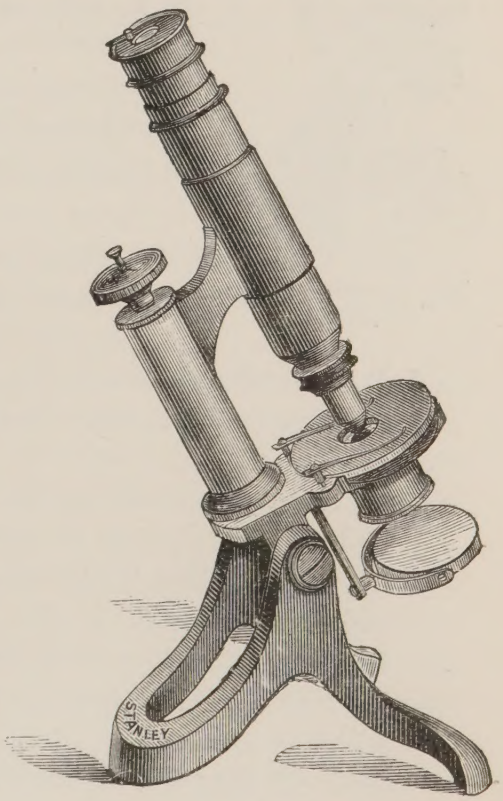

Fig. 8.—Student's Microscope.

Several makers have introduced microscopes even cheaper than the ones above-mentioned. Beck's Star Microscope, at *three to four* guineas, is a marvel of cheapness. Crouch and Parkes also make microscopes at £3 10s. to £4. It is requisite also to mention here, that Leitz, of Wetzlar, supplies a microscope at £5 5s. (stand No. IV., with lenses 3, 5, 7, oculars I. and III.), and one at £3 10s. (stand No. V., lenses 3 and 7, and oculars I. and III.), quite suitable for students' use.

Not a few prefer to purchase an English stand, with certain favourite German lenses. Those who propose to follow this course will find Baker's stand, and Zeiss's A and D lenses, a good

combination. Messrs. Baker (High Holborn) are also agents for Zeiss, Reichert, and other German firms.

It may appear unfair to mention the names of certain makers, and to omit the names of others. In a practical book, however, it is evident that *definite* advice should be tendered. We recommend the microscopes the performances of which we have had opportunities of judging. Other microscopes, of which we have had less experience, may possibly be as good as those recommended.

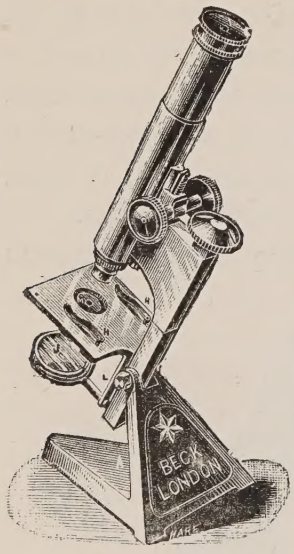

Fig. 9.—Star Microscope.

Of more expensive microscopes, in addition to several made by the before-mentioned firms, the following are recommended as suitable for advanced students :

Zeiss (Jena).—Stand No. V. *a*, with objectives A and D, and No. 3 ocular, costs £8 8s. Stand No. VIII., with similar objectives and ocular, costs £6 5s.

Leitz (Wetzlar).—Stand No. II., with objectives 3 and 7, and two oculars, costs £7 10s.; and with No. 8 objective as well, £9 5s.

Reichert (Wien).—Stand No. XI.*c*, with objectives 3 and 6, and one ocular, costs £7 19s.

Nachet (17, Rue Saint Severin, Paris).—No. IX., with objectives 3 and 6, and two oculars, is an excellent instrument, and costs £6 10s. We are very pleased with his No. XVIII., *microscope portatif de voyage*, the cost of which is £8.

Verick (2, Rue de la Parchemenaire, Paris) microscopes are good, but expensive.

Hartnack's microscopes do not seem to be so popular as they formerly were in this country, since the business of the old firm has passed into other hands. We have not had much experience, of late years, of this model, which was formerly so excellent.

As regards the higher class microscopes of many of the

English makers, the prices appear to be too high, and there is a tendency to make the instrument cumbersome.

The more expensive Stands and Lenses.—A few words about Zeiss's stands may be useful. Nos. I., II., III., and IV., price £15, £12 10s., £10 10s., and £10 5s., respectively, are provided with an Abbe's condenser, rack and pinion coarse adjustment, and all requisites for fine work. The eye-pieces are numbered 1—5, from two inches to seven-tenths of an inch focus, and cost seven shillings each ; the most useful numbers are 2 and 4. The objectives, A—F (twelve in number), vary in price from six shillings to £4 4s.; and oil immersions, $\frac{1}{8}$-inch, £12 ; $\frac{1}{12}$-inch, £16 ; and $\frac{1}{18}$-inch, £20.

Leitz's large stands are good and less expensive, about a third less in price than those described above. His oculars cost five shillings each ; and his oil immersions, $\frac{1}{12}$-inch, £5 ; $\frac{1}{16}$-inch, £7 10s. ; and $\frac{1}{20}$-inch, £10.

Reichert's stands and oculars are much the same in price as Zeiss's. His oil immersions, $\frac{1}{15}$-inch, £8 ; $\frac{1}{20}$-inch, £13 ; and $\frac{1}{30}$-inch, £18.

Powell and Leland's lenses are said to be the best in the trade, but are expensive. Their apochromatic homogeneous immersion object-glasses, $\frac{1}{8}$-inch, $\frac{1}{10}$-inch, $\frac{1}{12}$-inch, 1·40 numerical aperture, cost £25 each ; and a set of three compensating eye-pieces costs £5.

A **nose-piece** is a convenient appliance screwed to the microscope tube for carrying two or more objectives, so that one may be exchanged for another without the trouble of unscrewing it from the tube. The nose-piece, represented as attached to the microscope in Fig. 2, is for two powers. It is fixed to the tube of the microscope by a screw. The arm carrying the objectives revolves on a centre, which also attaches it to the upper portion or framework of the nose-piece, which is screwed into the microscope tube at one end, and is bent away from the stage on the other.

By carefully pushing one objective away from the microscope tube, the second one is brought into its place.

MICROSCOPICAL METHODS.

The second portion of our subject treats of the methods by which tissues should be prepared for microscopical examination. These methods will be described under two heads :

1. The Examination of Fresh Tissues.
2. The Examination of Hardened and Prepared Tissues.

1. The Examination of Fresh Tissues.

The simplest example of examining a fresh tissue would be afforded by the examination of a drop of blood. The drop is placed upon a thin piece of glass, about $\frac{5}{8}$ inch in diameter, or **cover-glass**, which is then inverted upon a glass slide or **object-glass**. The specimen of blood is at once fit for examination, and continues to be so until it dries up.

Similarly, one may examine fluid drops of any kind which are supposed to contain solid particles, the nature of which it is necessary to make out with the microscope ; *e.g.*, sediments of urine, scrapings of fresh tissues or organs, or micro-organisms when suspended in water. The only requisite to the successful examination being, that the particles should be thin enough to be transparent.

In some cases, again, it is requisite to examine some peculiarity of the structure of a tissue unaltered by reagents, or to observe some action, such as the movement of cilia, which speedily ceases after removal of the tissue from the body. Under such circumstances a small piece may be snipped off with scissors, teased (p. 40) with needles, and mounted in one of the following solutions, when sufficient of its structure can generally be made out—drying of the specimen being prevented by edging the cover-glass with oil or paraffin-wax :

Normal saline solution—a 0·6 per cent. solution of sodium chloride.

Blood serum.

Aqueous humour.

Iodised serum,—*i.e.*, serum or liquor amnii to which a little iodine has been added as a preservative.

Muscle may be examined without the addition of any

reagent ; but its evaporation must be prevented by the employment of appropriate means.

Other examples of a like nature will be treated of under their proper headings.

2. The Examination of Hardened and Prepared Tissues.

Speaking generally, it is impossible to make out the structure of a solid tissue thoroughly until a thin section of it has been obtained. The consistence of the tissues in the recent state, as a rule, will not allow of such sections being cut, and it is therefore necessary to harden them before attempting that process.

The hardening of tissues may be accomplished either by freezing them or by various chemical reagents. The latter act by coagulating the albumen, by withdrawing the water, or in some instances, perhaps, by combining with the albumen to form a harder compound—in a manner comparable to the process of tanning.

Hardening reagents.—It is of the utmost consequence that the process of hardening be attended to with the greatest attention. The choice of a suitable reagent in each case will be found, in spite of anything said to the contrary, a matter of considerable moment, and its mode of use equally important. We mention the hardening reagents we have found most serviceable.

Chromic Acid and Spirit.—A mixture of chromic acid and spirit is the fluid recommended for general use, and can be employed almost universally. It is thus prepared : Chromic acid $\frac{1}{6}$ per cent. solution (*i.e.*, containing 1 grm. in 600 cc. distilled water), 2 parts ; methylated spirit, 1 part.

Another way of making a solution is by taking equal parts of 0·5 per cent. solution of chromic acid and of methylated spirit. The mixture produces its effects in about seven to ten days.

Chromic acid, without the addition of spirit, in ·25 to ·5 per cent. solutions, is a rapid hardening fluid. The tissue is sufficiently hard in a week for sections to be made ; if, however, it remains in the mixture for a longer time, it tends to become brittle.

Potassium bichromate in solutions, the strengths of which vary from 1 per cent. to 5 per cent.: the best is the 2 per cent., and this, if changed every four days, hardens tissues in a fortnight.

Ammonium chromate in solutions 2 per cent. to 5 per cent. is superior in some ways to the similar potassium salt. Tissues should not remain in it for more than a day or two.

Ammonium bichromate is specially recommended for hardening the brain and spinal cord, in solutions of 5 per cent. strength. It must be prepared fresh when wanted.

Müller's fluid is made by taking potassium bichromate, 2 grms., and sodium sulphate 1 grm., and dissolving them in 100 cc. of distilled water. When this reagent is employed, the process of hardening is slow, but efficient. The fluid has the advantage of being exceedingly penetrating, and so will harden satisfactorily larger pieces of tissue than other similar agents. Half the quantity of copper sulphate may be substituted for the sodium sulphate.

Picric acid, in saturated solutions, or in mixture with an equal quantity of methylated spirit, or with an equal quantity of 5 to 10 per cent. hydrochloric or nitric acid, is a useful and quick-hardening fluid.

Mercuric chloride, in saturated or weaker solutions, or mixed with acetic acid, is becoming a popular hardening reagent. Its action is rapid.

Nitric acid, 3 per cent. to 10 per cent., is recommended for hardening the brain. It requires for its action two or three weeks.

Methylated spirit is a hardening reagent in very common use, and may be employed with advantage in the preparation of salivary glands, stomach and intestine, etc.

Absolute alcohol of specific gravity 0·795 is the most rapid hardening fluid. It is not often used, on account of its expense, and because it is said to have a greater tendency to produce shrinking of the tissue hardened, than preparations of chromium. The pancreas is an organ, at any rate, which must be immersed in this fluid, in preference to any other, for the purpose of hardening.

Osmic acid, 0·1 to 1 per cent.
Gold chloride, ·25 to ·5 per cent.
Palladium chloride, ·25 to ·5 per cent.

All of these solutions harden, and at the same time stain tissues.

Directions for Hardening.—Never use anything but fresh tissue. Nearly all the material used, therefore, must be taken from the lower animals just killed, as it is seldom possible to obtain specimens from the *post-mortem* room sufficiently fresh to give satisfactory results.

Cut the tissue into pieces with a sharp knife or razor. The size of the pieces will vary with the reagent used. When chromic acid is the hardening fluid, the pieces should not be larger than a hazel-nut. When alcohol, potassium bichromate, or Müller's fluid is employed, they should not exceed twice that size.

Do not wash with water; but if it be necessary to get rid of any foreign body, allow a small stream of saline solution, or of dilute spirit, or of a weak solution of potassium bichromate, to run upon the tissue from a wash-bottle.

Place the cut pieces in a large excess of the hardening reagent in a stoppered bottle.

Change the hardening reagent frequently—*e.g.*, the chromic acid and spirit solution on the second, fourth, and seventh days.

In all cases, in a week to ten days **remove the specimens to spirit**, or, if that fluid be contra-indicated, to a ·5 per cent. potassium bichromate to complete the hardening.

SECTION CUTTING.

Having hardened the material, the next thing is to cut *thin* sections from it; for unless the sections be thin, no amount of after preparation will make them fit objects for microscopical investigation. The methods which have been proposed from time to time to effect this object are numerous, but they may be divided into two classes—viz.:

(1) Methods of cutting by hand.
(2) Methods of cutting with machines called microtomes.

1. Section-cutting by Hand.

In cutting a small piece of tissue it is customary to embed it in some other tissue, or in a wax mass of some kind. For the former purpose, the tissue which is to be cut is placed between two pieces of hard liver,* or material of similar consistence, and held tightly in place between the finger and thumb, and cut with a razor in the manner to be described below. Instead of the liver, pieces of turnip, carrot, or potato may occasionally be substituted.

The usual method, however, is to embed the specimen in a wax mass.

Embedding materials are wax masses of some kind, modified

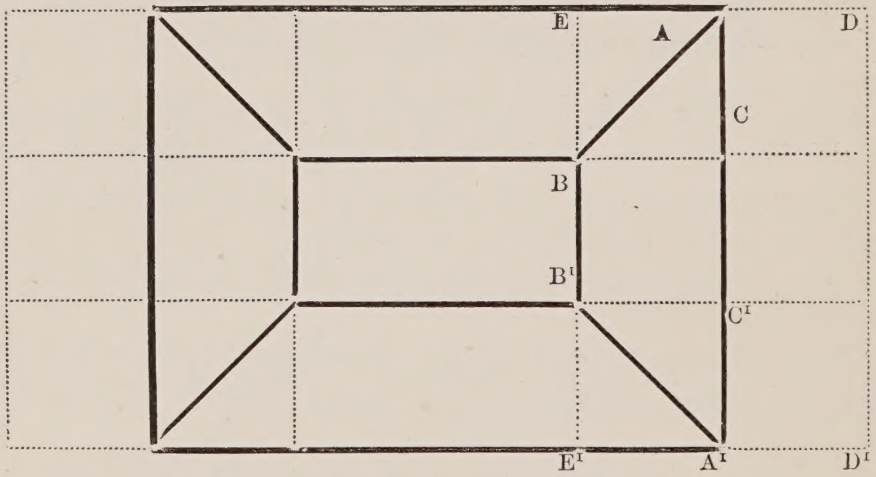

Fig. 10.—Diagram to show Formation of Embedding Box.

according to the state of the weather and the material to be cut. The following are those most commonly employed :

White wax and olive oil, equal parts ; melted and well mixed. This mass may be varied in consistency by varying the amount of the olive oil used. Japanese wax (8d. per lb.) is about one-fourth the price of English, and answers well.

Paraffin and Lard.—Take five parts by weight of solid paraffin (a paraffin candle will do very well), and one part by weight of hog's lard and of paraffin oil ; melt at a gentle heat, and mix thoroughly. Paraffin wax (1s. 4d. per lb.) of two kinds, melting at about 43° and 55° C., may

be obtained ; and, by mixing in various proportions, will supply wax of required melting-points for the different seasons of the year, without further admixture of lard.

Spermaceti and Castor Oil.—Take four parts of spermaceti and one part of castor oil.

Cacao butter may be used alone or combined with paraffin, wax, and oil, or with spermaceti and paraffin.

To melt the Wax Mass.—The wax mass is melted in a small porcelain capsule provided with a handle, over the flame of a Bunsen's burner or spirit-lamp. Care must be taken that the material is not burnt. It is usual in laboratories to place the capsule on a piece of fine iron gauze on a tripod, and to place a gas flame from a Bunsen's burner of the smallest size beneath it ; a glass rod may be used as a stirrer.

To embed the Specimen in Wax Mass for Cutting.—A piece of stout paper is taken, six inches long and three broad. This

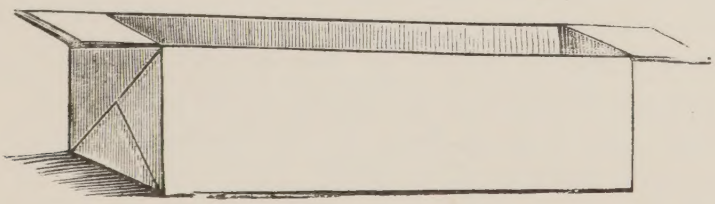

Fig. 11.—Paper Box for Embedding.

is doubled into three longitudinal folds ; after this from each end folds of two inches long are marked off. The paper is then opened out, and of the three longitudinal folds the middle one forms the bottom and the lateral ones the sides of the paper box. The ends are made from the middle part of the end folds. The ends of each flap are marked off into two equal squares, E C, C D, E^1 C^1, C^1 D^1. The squares E B A C and E^1 B^1 A^1 C^1 are doubled into two parts across the diameters A B, A^1 B^1, and these triangular folds thus made are pinched up and pressed against the end of the box to support it ; they are retained in position by the remainder of the end fold represented by A A^1 D D^1 being turned back over them.

Having made a paper box in the manner above described, and having melted the wax mass, take the specimen to be embedded

upon a needle mounted in a holder, and having removed the superfluous absolute alcohol (in which the tissue ought to have been immersed for at least ten or fifteen minutes before the operation is commenced) with blotting-paper, half fill the paper box with the melted wax mass, and dip the specimen into it several times, until it is thoroughly enveloped in wax. Allow the wax to cool, and place the tissue on it in the box at one end; then fill the box with melted wax, and after it has hardened, mark on the outside the position of the tissue. When quite hard, turn out the wax and the embedded tissue by opening the ends of the box, and place for a few minutes in methylated spirit.

Instead of the simple paper box, tin boxes with removable bottoms may be substituted, or hollow brass cylinders of various diameters, placed upon a glass plate.

The tissue is now ready for cutting. It is as well to shave off the corners of the wax, and also to cut off several thin slices from the end near which the specimen is with a sharp knife, in order that when the cutting of the specimen is to be done, the razor may not be blunted by having to cut much wax.

Razors.—As a good deal of the success of hand-cutting depends upon the sharpness of the razor, one cannot be too particular in choosing the instrument. It should be of good steel, capable of easy sharpening on a strop. It must also be broad bladed and hollow-ground. The original 'Army Razor' answers all these requirements. It should always be stropped in one direction. **Section-cutters** of various forms have been introduced, but do not possess any advantage over razors.

Directions for Hand Section-cutting.—Be sure the razor is very sharp.

Hold the razor firmly in the right hand, with the fingers closed above the handle; take the wax mass between the index-finger and thumb of the left hand, support the back of the razor on the former, and cut from left to right and from heel to tip of the razor.

Let the handle be kept in line with the blade.

Keep the blade well wetted with spirit, into a glass capsule half full of which also the cut specimens must be floated off with

a camel's-hair brush after each sweep of the razor ; unless the specimen has been already stained and dehydrated, in which case clove oil is to be used instead of spirit for wetting the razor and preserving the sections.

2. Section-Cutting with Microtomes.

It must be confessed that hand section-cutting is very much going out of fashion.

The reason is not far to seek. The skill required for hand cutting in order that sections uniformly thin may be cut with certainty is very rare. Microtomes by various mechanical means in cutting sections do away with the uncertainty of hand cutting. They are invariably used when a large number of sections is required, and are invaluable for cutting for a large class. Some histologists still prefer hand cutting for all delicate materials ; indeed, for some organs few microtomes can be employed with satisfactory results.

The simplest microtome is **Ranvier's**, which consists of three hollow brass cylinders fitting one inside the other ; to the most external a flat circular brass plate is fixed at one end, and to the other a cap which can be screwed on, carrying through its centre a fine screw. The tissue having been embedded in paraffin-wax in one of the cylinders and allowed to cool, the cap is adjusted, the microtome held in the hand, and the screw turned until the wax is carried up sufficiently high for the embedded specimen to be cut ; the flat plate guides the razor. After each section is cut the screw is turned slightly, thereby presenting sufficient of the specimen for another section.

Stirling's microtome is on the same principle as Ranvier's, but is larger, and is fixed to a table by means of a screw. Only one cylinder, however, is fitted to the instrument.

Rutherford's microtome provides a trough which may be used to contain a freezing mixture of ice and salt. When this is used, the tissue is embedded in thick gum, which, on freezing, becomes quite solid, and may be readily cut.

Both these microtomes may be provided with glass plates instead of brass, to guide the razor.

These three microtomes raise the tissue embedded in its

dara ffin or gummy cylinder to the knife. This is, in our opinion, a mistake. The tissue should be firmly fixed, or at any rate should be exposed to as little disturbance as possible, and the razor should be lowered to it, and not *vice versâ*.

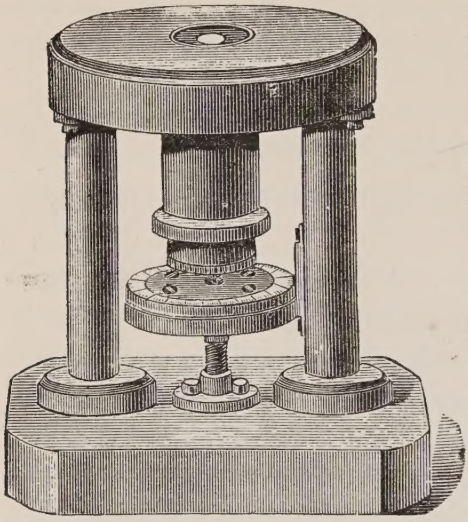

Fig. 12.—Zeiss's Microtome.

In **Zeiss's** microtome (Fig. 12) our objection is partly met by the cylinder containing the wax being moved upwards bodily, and not the wax only. For many reasons, indeed, we strongly recommend this form to students. The instrument is well made, and the screw is so fine that sections of extreme thinness may be cut by its aid. The price of it is £2.

Of microtomes upon the same principle, but in which the knife is fixed and works in a plane, moving backwards and forwards, and cutting a section at each sweep, the tissue embedded in wax or frozen in gum being raised by means of a fine screw, we may mention the following :

Rivet, the £2 10s. form is the best.
Jung (£7, £4 15s. and £2 15s.), Heidelberg, of extreme delicacy and accuracy.
Leitz, Support-Mikrotom (£6).
Reichert, Schlitten-Mikrotom (£5 12s., £7 10s., and £9 5s.).
Bœcker, Wetzlar (£9 9s.).
Zeiss (£5 10s.).
Beck, Universal Microtome (£15 15s.).
Caldwell's Microtome, and the rocking-microtome, made by the Cambridge Instrument Company.

All of these are good. Special advantages are usually claimed for each variety—some automatic action of turning the raising screw, some extra steadiness, a ribbon arrangement by which the sections when cut are removed in a continuous series, or what not.

At the same time it should be recollected they are very expensive —Caldwell's microtome originally costing, we believe, about £30. The ribbon microtomes are indeed triumphs of mechanical construction, but are, of course, not applicable for students' use.

The form of microtome, however, which we believe from long experience to be the best for all practical purposes is Williams's. In it the specimen is fixed, and the knife is approached to it. It consists of a circular wooden box about eight or nine inches in diameter. Into the centre is fixed a circular solid brass pillar, with a brass plate at the top. This box contains a freezing mixture of ice and salt, and is provided with a waste tube to get rid of the water as the ice melts. The cover of the box is wooden, but has a plate of glass fixed upon its upper surface ; it also has a central aperture to admit the top of the brass cylinder. This cover is fixed down after the freezing mixture has been placed in the box, and fastened with a lateral screw. The specimen, which should first be left for twelve hours in water (to get rid of the spirit), is placed on the cylinder plate in a little gum. The gum is soon frozen, and the specimen is fixed by this means. The cutting is effected by means of a razor, which is fixed in a movable triangular brass frame, supported on three screws. By the adjustment of the front screw the thinness of the section is regulated. The frame works smoothly over the glass table. The upper surface of the razor should be slightly moistened with gum. As the sections are cut they should be swept off the razor with a camel's-hair brush, and should be washed in warm water before staining.

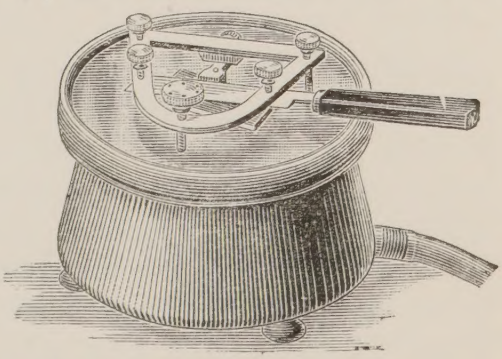

Fig. 13.—Williams's Microtome.

This microtome adapted for use, with **ether** as the freezing agent, may be obtained in various forms. The instrument costs about £2 10s.

Freezing mixtures are made by taking pounded ice, adding some rough bay-salt, and thoroughly incorporating the one with

the other. In very cold weather snow may be obtained and used in place of the ice.

A strong **solution of pure gum arabic** in boiling distilled water is made, and filtered through calico. To this are added a few drops of melted pure carbolic acid. The proportion of gum is about a pound to a quart of water. It should be kept in a stoppered bottle, and boiled occasionally, to prevent decomposition.

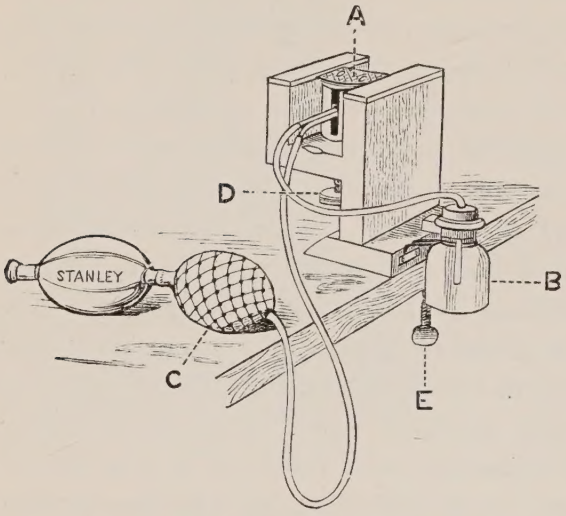

Fig. 14.—Cathcart's Microtome.

A Metal plate upon which the tissue is placed in gum; B Bottle containing ether; C ether spray apparatus; D Screw to raise tissue when frozen; E Screw to fix microtome to table. The cutting is done by a plane which rests on the supports on either side of A.

We may mention a convenient and cheap form of ether-freezing microtome, viz., Cathcart's, costing 15s. to 20s., a figure of which we annex.

We now adapt the principle of the knife-carrying triangle in section-cutting to nearly all our histological work. It was found in practice that the small brass caps for use with specimens embedded in wax, to be screwed on to the central brass pillar in Williams's microtome, did not work well. Instead of this, we use a sheet of plate-glass, upon which we place the specimen to be cut thoroughly infiltrated with paraffin-wax. When the wax is cold the specimen is fixed upon the plate. The triangle in use in this case is of much heavier make, and the screws are larger, and of finer worm, than usual. With a simple apparatus such as this, sections of every kind of tissue which will bear staining in mass and soaking in melted wax may be made with ease. We have had much success with embryological specimens treated after this manner. Mr. Stanley has made these heavier triangles for us. Their cost is about £1 5s.

As regards the preparation of tissues for this method of cutting, see p. 41.

Having cut thin sections of the tissue to be examined with the microscope, and having washed them in water to get rid of the gum, or in spirit in which the adhering wax drops off if the specimens have been embedded in that material, it is necessary to proceed to the next operation, viz., that of **Staining.**

STAINING REAGENTS.

Almost all tissues require to be stained with some dye, in order that certain elements of their structure, which would otherwise be indistinct, may be more clearly shown. The following dyes are those which are usually employed :

Aqueous Hæmatoxylin or Logwood is the simplest and best. It is a highly selective and clear dye. It is made by taking dried extract of hæmatoxylin 60 grms., alum in powder 180 grms., and rubbing thoroughly together in a mortar, and adding slowly 300 cc. distilled water ; mixing carefully and filtering ; to the filtrate adding 20 cc. of absolute alcohol, or 50 cc. of a 1 per cent. solution of thymol, and preserving in a stoppered bottle.

The solution should be kept in a cool place for at least a fortnight before using. The older it is the more excellent it becomes. A second but weaker solution may be made by adding a second 300 cc. of distilled water to the same logwood alum, and proceeding in a similar manner.

Alcoholic Hæmatoxylin is prepared by making saturated solutions of crystallized calcium chloride and of alum in proof spirit, mixing the solutions in the proportion of one to eight, and adding to this mixture a saturated solution of hæmatoxylin in absolute alcohol, drop by drop, until the whole becomes a dark purple. This solution may be used at once, but is greatly improved by keeping.

Carmine was formerly used much more than it is at present ; it has the disadvantage of staining specimens a colour trying to the eye, whilst it has not the great selective power of hæmatoxylin. There are many ways of making carmine solutions.

(*a*) Take 2 grms. of carmine, and rub thoroughly in a mortar with a few drops of water ; then add 4 cc. liq. ammoniæ and 48 cc. distilled water ; filter into a bottle, which should be left unstoppered for a day or two for the excess of ammonia to evaporate. This forms a strong solution, which must be diluted before using (Klein).

(*b*) Beale's solution is thus prepared : Dissolve carmine grm. j. in liq. ammoniæ fort. 3 cc. warm, add aq. destillat. 120 cc., and filter. Then add glycerine 30 cc., and spir. vini rectif. 120 cc., and keep in a well-stoppered bottle.

(*c*) Borax carmine is made by thoroughly mixing carmine (grms. ij.) and borax (grms. viij.) in a mortar, and dissolving in warm water for twenty-four hours. The supernatant fluid, which should be decanted, is then ready for use.

(*d*) Alum carmine. Boil an aqueous solution of ammonium alum with excess of carmine for ten to twenty minutes ; filter, and dilute the filtrate until it contains 1 to 5 per cent. of alum. Add a few drops of carbolic acid to prevent the growth of fungus.

(*e*) Alcohol carmine. A teaspoonful of carmine dissolved by heating for about ten minutes in 50 cc. of 60 to 80 per cent. alcohol, to which three or four drops of hydrochloric acid have been added, and then filtered.

Picrocarmin, *or solution of picrocarminate of ammonia*, is now much used, in combination with another dye when it is expedient to 'double stain' tissues in order to bring out certain special features in their structure. It is prepared by adding a saturated ammoniacal solution of carmine to a saturated solution of picric acid until a precipitate begins to form, evaporating in a water-bath to one-third its bulk—filtering and evaporating the filtrate to dryness : a crystallized mass, easily soluble in water, is obtained, which is picrocarmin. The strength of the solution should be about 1 per cent. to 3 per cent. During preparation the ammonia should be kept in excess.

It may also be made by taking Beale's carmine without

alcohol, and adding the picric acid in a similar manner. The glycerine prevents burning, which is not unlikely to occur.

Many other formulæ have been given for making this useful dye, which we do not find superior to those above given.

Cochineal.—(*a*) Take 7 grms. cochineal and 7 grms. alum in powder, thoroughly rub together in a mortar, and add 700 cc. distilled water; evaporate to 400 cc., filter *twice*, and afterwards add ʒj. or ʒij. absolute alcohol, or of 1 per cent. thymol solution.

(*b*) Powdered cochineal 10 grms., alcohol 70 per cent. 100 cc. Macerate for a week, in a stoppered bottle, shake up frequently. The filtered solution will be ready for staining. Wash stained sections in alcohol of above strength.

Purpurin (Ranvier).—The formula of a solution of this dye first introduced by Ranvier, which is most stable, is Grenacker's: Glycerin 50 cc., powdered alum 2 grms. Add a knife-pointful of purpurin, and boil. Let the solution stand for several days, and filter. Staining in this solution takes 10 to 30 minutes.

Ink-stain.—Excellent results may be obtained by staining nervous tissue, especially the spinal cord, in diluted solutions of Stephens's blue-black ink.

Eosin, $C_{20}H_6Br_4O_5K_2$, a rose-red phenol dye, is used chiefly to stain nervous tissue and blood, and in combination as a double stain. It is very soluble in water, and requires to be fixed. In practice it is advisable to leave the tissue in a ·01 per cent. solution for twenty-four hours, and then to pass it through acidulated water. It is unnecessary to keep it in solution, as it can be made so easily.

Some, however, recommend a 1 per cent. solution in alcohol, others in ammonia, and others 1 per cent. in alcohol with alum. As far as our experience goes we have obtained the best results from a simple aqueous solution.

Aniline Dyes.—An immense number of aniline dyes are now manufactured, and most of them have been tried in histological work.

Many of them stain quickly and penetrate well, but their usefulness is diminished by their tendency to wash out.

TABLE OF THE CHIEF ANILINE DYES WITH THEIR SOLUBILITIES.*

Brown.	Red.	Orange.	Yellow.	Green.	Blue.	Violet.
Bismark—partially sol. in water, sol. in dilute spirit.	**Aniline Scarlet** — insol. in water, freely sol. in methylated spirit.	**Aurin** — insol. in water, fairly sol. in absolute alcohol.	**Fluorescin**—greenish-yellow: insol. in water, sol. in spirit, the solution being beautifully fluorescent.	**Iodine green** — blue-green: freely sol. in water or spirit.	**Soluble Aniline blue** — freely sol. in water.	**Hoffman's Violet** — freely sol. in water and in dilute spirit.
Vesuvin—sol. in water.	**Flamingo** — deep brownish-red: partly sol. in water, freely sol. in spirit.	**Aniline orange**—insol. in water, fairly sol. in absolute alcohol.	**Aniline primrose**—only partially sol. in spirit.	**Malachite green** — a less blue-green: freely sol. in water and in spirit.	**Bleu de Lyon** — insol. in water, freely so in strong spirit.	**Methyl Violet** — the red predominating: sol. in water partially, freely sol. in spirit.
Chrysoidin—sol. in water.	**Ponceau**—deep red, crimson: partly sol. in water, freely in methylated spirit.	**Tropœolin** — in deep yellow glistening scales: partly sol. in water, more so in methylated spirit.			**Methylen blue** — deep blue: freely sol. in water and in spirit.	**Gentian Violet** — the blue predominating: freely sol. in water.
	Rosanilin — partly sol. in water, freely in dilute spirit.	**Phosphin**—yellowish-orange: partially sol. in water; more so, but not freely, in spirit.			**China Blue**—freely sol. in water.	**Tyrian Blue**—near to violet: sol. in water.
	Fuchsin — partly sol. in water, sol. in dilute spirit.	**Safranin**—sol. in water and in spirit.			**Serge Blue**—freely sol. in water.	**Spiller's Purple**—sol. in spirit, partially sol. in water.
					Blue Black—freely sol. in water.	

* Taken from *Quarterly Journal of Microscopical Science*, vol. xxiii., p. 291. 'On Staining . . . with Aniline Dyes,' by V. D. Harris, M.D.

In using aniline dyes, therefore, it is necessary to stain specimens deeply, and to pass them through the desiccating and clearing fluids quickly, otherwise the colour passes into the solutions ; for at present no one fixing fluid has been made which produces certain results ; in some cases, however, a weak solution (about ·5 per cent.) of hydrochloric acid is effectual. Solutions of mercuric chloride, of potassium acetate, of sodium xanthate, and of tannin, have also been recommended as mordants.

The table on p. 30 includes most of the aniline dyes which have been employed in microscopical work.

Speaking roughly, the dyes are most useful which will dissolve without the aid of spirit. In nearly every case slow is to be preferred to quick staining ; this is especially true with regard to iodine green, aniline black, and Bismark brown.

Some information will be given later on as to the use of these anilines in bacteria staining.

Silver Nitrate is used when it is required to demonstrate the endothelial cells of serous membranes. The salt is taken up by the intercellular substance when fresh, and is reduced as a black precipitate under the action of light, which maps out the cells in black lines. The fresh tissue should be placed after removal from the body in a ·5 or ·25 per cent. solution for ten or fifteen minutes ; it should then be washed carefully in distilled water, and exposed to the light in glycerine diluted with three times its bulk of distilled water. Silver nitrate is also used to stain nerve-fibres.

Preparation of the Solution of Silver Nitrate.—Powder 5 grms. of crystallized silver nitrate finely in a mortar, and add gradually 1000 cc. cold distilled water. After the salt has dissolved, preserve in a stoppered bottle of dark glass, or in one around which some black paper has been pasted, and keep in a dark cupboard. The use of the solid silver nitrate in bringing into view the cell spaces of the cornea will be alluded to farther on.

Ammonium-molybdate produces a bluish-gray general stain, which acts well as a base for double staining. A 5 per cent. solution in water may be used, and the specimens should be exposed to the light for twenty-four hours. The salt is expensive, and the advantage of its use not very marked.

Gold chloride selects and stains certain tissues, principally the nervous ; it also brings out the cells of fibrous connective tissue, cartilage and cornea.

Method of Gold Staining.—Of the many ways we have tried of using this salt we recommend the following. The tissue is removed from the animal immediately after it has been killed, and is placed in ·5 per cent. solution of gold chloride for half an hour to an hour ; it should then be washed in distilled water, kept in a *warm*, dark place in a saturated solution of tartaric acid for three or four hours, and afterwards exposed to the light in equal parts of glycerine and water. Immersion in filtered lemon-juice, in citric acid, or in dilute formic acid for about five to ten minutes previous to the bath of gold chloride is advised by some. Various acids, too, are recommended instead of tartaric, as citric (saturated solution), formic (20 per cent.), acetic (4 per cent.), oxalic ($\frac{1}{2}$ per cent.). The double chloride of potassium and gold may be substituted for the gold chloride.

Method of preparing the Solution.—The gold salt is sold in sealed glass tubes, containing about 1 grm. ; the tube should be broken, and the salt should be dissolved and preserved in a manner similar to that described above under the heading of Silver-nitrate Staining.

Palladium chloride, an irregular general stain, yellow to black, in solutions varying from ·1 to ·05 per cent., may be used ; it has the same effect as gold chloride in hardening tissues and staining them at the same time. It is very expensive, and is not recommended.

Osmic acid, as well as the two preceding salts, possesses the property of hardening as well as staining tissues placed in it. It is usually sold in 1 per cent. solutions, which may be diluted with distilled water at pleasure. The solution must be kept in a dark glass bottle. Osmic acid stains fat-globules black, and *brings out* the medullary sheath of nerves. Specimens to be stained with this reagent must remain in it for about an hour, and should then be removed to spirit.

Method of using the Staining Reagent.—Unless another reagent is specially advised, the student should, as a matter of

course, employ hæmatoxylin, as it is the easiest to use and the best of all stains.

Such specimens as have been already selected as sufficiently thin should be thoroughly washed in distilled water or in methylated spirit, and placed in spirit in a watch-glass of suitable capacity.

Sections of tissues, hardened in any chromium preparation, should then be transferred to a watch-glass containing a solution of sodium bicarbonate 1 per cent., and should be allowed to remain in it for five minutes, in order that the effect of the hardening reagent may be neutralized. They should then be well washed in warm distilled water (30° to 40° C.).

To prepare the staining solution, it is as well to take a large watch-glass, fill it three-quarters full of distilled water, and then add to it from five to ten drops of aqueous hæmatoxylin. The hæmatoxylin solution is kept in a bottle provided with a funnel and filter-paper, as the reagent must be filtered before it is used.

Having made the solution, and thoroughly mixed it, place the sections in it carefully one by one with a broad needle, and if they float, press them down and leave them in the fluid for some minutes. The time required varies, as some tissues stain much quicker than others, and it is therefore necessary to take out a section from time to time, and to place it in a watch-glass full of distilled water, so that the staining may be regulated. Great care is necessary, in order that the specimens be stained neither too much nor too little. The examination of the specimen in the watch-glass should be done with a white background, which may be obtained by placing the glass on a white filter-paper, or still better on a white glazed tile. This tile may easily be obtained, and will be found a great convenience.

It may not be out of place to notice here that stained specimens should always be examined during manipulation in this manner, and that unstained specimens can be most conveniently examined if placed in a watch-glass upon a black plate. This black plate is generally a square piece of glass with the back blackened and protected with varnish. A dark-coloured glazed tile will answer the purpose.

When the sections are sufficiently stained, they must be

washed in distilled water and placed in methylated spirit, ready for the next process (pp. 35-40).

Instead of the washing in the warm distilled water previous to staining, washing in a very dilute solution of hæmatoxylin (three or four drops to a watch-glassful of water) may be substituted.

If the sections be accidentally overstained, place them in a ·5 per cent. solution hydrochloric acid for a few seconds.

Watch-glasses and capsules of various sizes should be obtained. The small thin shallow glasses are of no use; those recommended are large, thick, and deep. The capsule which is used to contain spirit for hand-cutting must be large and capacious—2½ to 3 inches in diameter, and 1½ inches deep.

Staining with Carmine, Eosin, and Aniline Dyes.—The method of staining with these reagents is very similar to hæmatoxylin staining, but specimens should remain in the solution for a much longer time (twenty-four hours or more), and the dye should be fixed by passing the sections through acidulated water. Sodæ bicarbonate solution is not necessary. When a spirit solution of any dye is used, pass rapidly on to the next process after staining. We cannot, however, give any other than general directions for staining with these dyes, and the operator will soon find out the best methods of using each.

Double Staining.—To demonstrate the structure of some tissues satisfactorily, it is necessary to make use of two or more staining reagents. This process is called *double* or *multiple* staining, according as two or more dyes are used. The simplest form of double staining is that adopted for tissues which are first stained with silver nitrate, gold chloride, or similar reagent, and then with hæmatoxylin, carmine, or aniline. Other combinations may be made, of which the following are useful :

Picrocarmin and hæmatoxylin.
Eosin and hæmatoxylin.
Eosin and aniline green, etc.
Picrocarmin and anilines, etc.
Fuchsine and methylin blue.
Eosin and methylin blue.
Aniline rose and aniline green.
Bismark brown and aniline green.

It is unnecessary to do more than mention *treble* staining. If this be ever required, the combination recommended is, (1) *picrocarmin;* (2) *rosein;* (3) *iodine green.*

As regards staining with four colours, one of the authors has found that it may be done with (1) *picrocarmin or eosin;* (2) *logwood;* (3) *aniline rose;* (4) *aniline green.* If the tissue have been already stained in gold chloride, five stains will have been used. However, the processes are tedious, and not of any great practical value.

Dehydration and Clearing.—When the sections of the tissue have been stained, or double stained, as the case may be, unless they are to be mounted in glycerine, or one of its substitutes, they require to be dehydrated and cleared.

Dehydration.—This process is effected by passing them through methylated spirit and absolute alcohol. After staining they should remain in each of these fluids for five minutes.

Clearing.—After immersion in alcohol, the sections have to be placed in a fluid which will render them transparent. The fluid most extensively employed is *clove oil:* it is a hydrocarbon isomeric with oil of turpentine, and possesses the advantage over the latter of being more agreeable to manipulate. Oil of turpentine may, however, be used, as also some of the other oils which are isomeric with it. A clearing fluid we have found a cheap substitute for clove oil is, turpentine 4 parts, creasote pure 1 part, mix and filter. These fluids penetrate the tissues, and render them fit to be mounted in Canada balsam.

MOUNTING.

For this process the operator must have—

(1) *Glass slides,** which are slips of glass three inches long and one inch broad, about the thickness of ordinary window-glass, with or without ground edges. They are sold by the makers of microscopic requisites, at from 2d. to 6d. a dozen. It

* These, as well as cover glasses, may be obtained of Tate, Holborn; Stanley, London Bridge; Beck, Cornhill; Medland, London Bridge; Baker, Holborn; Crouch, Barbican; and of many others.

is as well to keep a good stock on hand ; and, as a rule, within reasonable limits the thinnest are the best.

(2) *Cover-glasses*, which are made of extremely thin glass, circular or square, $\frac{1}{2}$ to $\frac{5}{8}$ inch in diameter. There are several kinds sold, usually known as ordinary, thin, and extra thin. The ordinary are quite thin enough for the student, but sometimes it is as well for him to provide himself with each kind. The ordinary cover glass measures from ·004 to ·008 inch in thickness.

To measure Cover-glasses.—Thin glass may be placed edgewise in the stage forceps of the microscope and measured very accurately with the micrometer. The student may, however, neglect this operation.

To cut Cover-glasses.—Thin glass may be bought in sheets, and cut into squares or circles by the operator with a diamond, but this is not advised.

To clean Slides and Cover-glasses.—For the former it is generally only necessary to wash them in soft water or weak soda-water, drying with a clean cloth, and polishing with chamois leather. A mixture of ether and alcohol, caustic potash, infusion of nut-galls or alcohol, however, has to be used sometimes. Cover-glasses may be washed in strong potash, or in infusion of nut-galls, or in alcohol, and should be dried and polished with a fine cambric pocket-handkerchief.

(3) *A section-lifter*, which may be easily made by beating out flat one end of a thick copper wire, four or five inches in length. The flattened portion should afterwards be filed at the edges, and rubbed smooth with sand-paper.

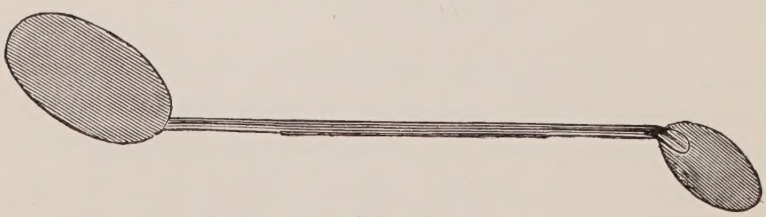

Fig. 15.—Section-lifter.

More carefully finished section-lifters may be obtained of instrument-makers ; the annexed figure represents one form

of lifter suggested by the authors for mounting large sections.

Mounting Fluids.—Fresh tissues may be mounted in any of the reagents mentioned at p. 16. The following fluids may also be used:

Potassium acetate, in saturated solution, employed chiefly for mounting vegetable tissues.

Glycerine, one of the most useful of mounting fluids. It may be employed for fresh tissues, as well as for those which have been hardened and cut into sections. The fluid should be of high specific gravity; some microscopists advise dilution with a third of distilled water, but we cannot endorse this recommendation.

It must be remembered that in this reagent some tissues swell up—*i.e.*, fresh tendons—and so lose their characteristic structure, and for such the reagent is contra-indicated.

Directions for Mounting in Glycerine.—Place the tissue for a quarter of an hour in distilled water, transfer to the slide, spread out, and remove the excess of water. Then place a drop of strong glycerine on a thin cover-glass, and invert it over the specimen, taking care to keep the drop in the centre of the cover until its lowest point touches the centre of the specimen, and then allow the cover to fall gently on it. If the glycerine entirely fills the space under the cover, and is not in excess, the edges may be painted round with some cementing varnish. If too much have been taken, the excess may be removed with a capillary pipette, or with filter-paper.

Farrant's solution is a useful substitute for glycerine, to be employed for the mounting and preserving of sections and teased specimens. It does not render the tissues so transparent as does glycerine; whilst the cover-glass becomes fixed to the slide as the solution hardens.

It is made by adding an equal weight of powdered gum arabic to a mixture of equal parts of glycerine and a saturated aqueous solution of arsenious acid.* The mixture is then

* The arsenious acid may be omitted, and a piece of camphor may be introduced in its place.

allowed to stand for six weeks, being stirred at intervals. Any gum which remains undissolved is then filtered off; and the resulting clear filtrate is Farrant's solution.

Glycerine jelly is made by taking pure gelatine 8 parts, soaking it in cold water for several hours, pouring off the water and warming the gelatine until melted, adding 1 part of egg albumen, boiling until the albumen is coagulated and the gelatine is clear, filtering through flannel, and finally adding 6 parts of a mixture of 1 part of glycerine to 2 of camphor water. [See also Appendix.] It is advisable to buy this reagent, as the making of it is difficult.

Carbolic acid solution (one in forty) may sometimes be used, or a mixture of this with alcohol and arsenious acid.

Castor oil is employed to mount crystals, etc., which are soluble in Canada balsam.

Directions for Mounting in Canada Balsam, etc.—It may be as well here to recapitulate the processes to be gone through before the sections are fit for mounting in Canada balsam. If the section be cut in spirit or water, it is passed through—

Sodæ bicarbon, 1 per cent.	If hardened in any preparation of chromium to neutralize the hardening reagent.
Distilled water.	To wash away excess of the bicarbonate.
Staining fluid.	
Methylated spirit. **Absolute alcohol.**	To dehydrate.
Clove oil, or **Turpentine and Creasote, etc**	To render transparent.

Preparation of Canada Balsam Solution.—The best method is to take the commercial balsam, expose it to a temperature of 70° C. for twelve hours, to dry it and render it quite hard, and then to dissolve in benzol and filter. It should be preserved in a stoppered bottle.

Another method is to mix equal parts of Canada balsam and chloroform and warm. The balsam is entirely dissolved. Filter.

Preparation of Dammar Solution.—Dissolve gum dammar in powder 50 grms. in 150 cc. turpentine, and filter ; gum mastiche 50 grms. in 200 cc. chloroform, and filter. Mix the solution, and again filter. This solution can be employed in place of Canada balsam. Benzol may be employed instead of turpentine and chloroform.

Drop-bottles for Canada Balsam, Dammar, etc.—Mounting fluids such as Canada balsam are usually kept in bottles with narrow elongations of the stopper, which dip into the fluid, and will deposit it on the cover-glass in drops (Fig. 16).

Having placed the sections in clove oil in a watch-glass or suitable glass dish upon a white plate, as above directed, take a clean slide and place it upon a white filter-paper. Next, by means of a section lifter and a needle, withdraw the section from the clove oil, and bring it down upon the centre of the slide, and remove the excess of clove oil with filter-paper. Then take a clean cover glass, and place a drop of the Canada balsam solution on the centre of it, and proceed in the manner described under the head of Glycerine Mounting (p. 37), or take hold of a corner with a pair of forceps, and gradually incline it over the speci-

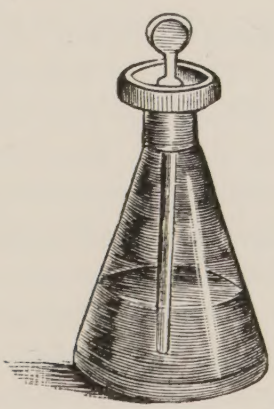

Fig. 16.—Drop-bottle for Canada Balsam, etc.

men, allowing the opposite edge to touch the slide first outside the specimen, and then allowing the other part held with the forceps to fall gently on the specimen. Either of these methods will prevent the formation of many air-bubbles under the cover-glass. It is almost impossible not to have some, but

these will disappear if the specimen be kept in a fairly warm place for a few hours.

Cementing Reagents.—Sections mounted in glycerine and in similar fluids must have, and those mounted in Canada balsam or Dammar varnish may have, their cover-glasses secured with cementing material which is painted on with a camel's-hair brush. It would be useless to mention more than a few of these cementing fluids. The most useful are :

Dammar varnish, prepared according to the above formula.

Canada balsam, made with benzol.

Brunswick black, which is asphalte dissolved in turpentine or naphtha.

Gold-size.

Marine Glue (Hollis's).—The authors find this the most convenient cement, as it dries very quickly, and is very insoluble.

Before concluding this portion of the introduction, we must not omit to mention certain additional operations of use to the microscopist.

TEASING

Is done with needles mounted in some kind of handle. These may be made very easily by taking a fine-pointed needle, heating the eye red hot in a flame, and pushing it into a penholder or suitably cut piece of wood. More elaborate needle-holders may be bought of any maker of microscopic requisites.

Directions for Teasing.—Cut off a small portion of the tissue to be teased with a sharp knife or pair of scissors, and place it on a clean glass slide, in a small drop of the fluid in which the object is to be mounted. Place the slide upon a firm support as close to the eye as convenient, and with a couple of mounted needles separate the tissue into, as nearly as possible, parallel fibres. Some tissues cannot be so separated, and in that case they must be broken up with needles into minute pieces. Sometimes the aid of a lens or of a dissecting microscrope has to be called in.

A rougher method than teasing may occasionally be used when epithelium cells have to be examined. It consists of

taking a scraping of the epithelial surface on a slide, adding a drop of glycerine, and gently tapping the cells with the smooth end of an ivory or bone penholder until they are seen under the microscope to be separate. This method is, however, almost sure to damage a certain proportion of the cells.

SOFTENING, OR DISSOCIATING.

This process is employed for two purposes—either to facilitate the teasing of tissues into their elements, or in order that sections may be conveniently cut of tissues otherwise too hard for the knife. Under these circumstances small pieces of the given tissue should be allowed to soak in one of the following solutions :

Potassium Bichromate, 2 per cent.—This is particularly useful for the purpose of dissolving up the cementing material between the fibres of tendon.

Baryta solution, for a similar purpose.

Iodized serum.

Weak methylated spirit, for lymphatic glands, spleen, and testicle.

Chromic acid, 5 per cent., to which a few drops of sulphuric and nitric acid have been added, for bone and teeth.

Hydrochloric acid, 2 per cent. to 3 per cent., or a mixture of this with nitric acid, 1 per cent., for bone, teeth, and cartilage.

Picric acid, a saturated solution, especially for teeth.

Of Preparing Sections of Brittle or Specially Delicate Material.—When tissues are too brittle and too delicate to be cut into sections, stained and subjected to the risk of damage by transference from one reagent to another in the ordinary way, it is advisable to stain them *en masse*, and so to prepare them that the sections when cut may be at once mounted. This method is specially to be adopted with embryological specimens, with the internal ear, and sometimes with diseased tissues. The process may be thus summarised. After hardening, the specimen is placed in some staining reagent which possesses the quality of penetrating as well as that of being stable—carmine, cochineal, or picrocarmin being the most suitable for the purpose. In this

solution it is kept for twenty-four to forty-eight hours, at a temperature of about 32° to 35° C. It should then be washed in water, next in methylated spirit, and afterwards in absolute alcohol for an hour at least. From this reagent it must be removed to turpentine for a variable time, according to the size of the specimen, for at least an hour. From turpentine, the excess of that reagent having been removed with blotting-paper, it should be placed in melted paraffin. The paraffin should be kept just melted at a temperature, say, of 45° to 55° C. The tissue must remain in melted paraffin for at least two hours, but a longer time is necessary if the size be large, and twenty hours is recommended if the time can be spared. After this the tissue may be placed upon the glass plate described on p. 26, and cut with the microtome there mentioned. After some practice ribbons of sections may be cut, and they may be mounted according to the useful 'shellac plan.' This consists in painting the slide upon which the sections are to be mounted with white shellac dissolved in creasote with a camel's-hair brush, and placing upon it the ribbon of sections. On slightly warming the slide the sections sink into the shellac, and by these means are firmly fixed to the slide. The paraffin can then be dissolved away by leaving the slide in turpentine for some time at the ordinary temperature. After the last operation has been completed the sections may be mounted in Canada balsam.

INJECTION.

In order to show the distribution of the blood-vessels in an organ, or of the lymph-vessels or channels, or to demonstrate the bile-ducts in the liver, methods of injecting these vessels, etc., are adopted. The material used is such that when the operation is over, it remains *in situ* within the vessels.

In carrying out this process, the injection material should first be prepared. It consists of a mixture of carmine and gelatine, made as follows: Suspend 4 grams of carmine in a minimum of water, adding 8 cc. of liq. ammoniæ, and afterwards 48 cc. of distilled water; filter the solution. Dissolve 13 grams of the best gelatine in 100 cc. of water by the aid of heat derived from a water-bath, and filter. Add the carmine solu-

tion to 72 cc. of the warmed gelatine filtrate. Then add, with constant stirring, 4 or 5 cc. of glacial acetic acid to each 18 cc. of the carmine and gelatine solution, kept at a temperature of 40° C.

A solution of gelatine prepared as above, with the substitution of Berlin blue for the carmine, forms an equally good injecting fluid. The Berlin blue is an aqueous solution of a strength of 2 per cent., made by dissolving 10 grams of Brücke's soluble blue in 500 cc. of distilled water.

Methods.—The animal to be injected should be killed by chloroform, in order that the arteries may be dilated to their utmost extent, and an incision into one of the larger blood-vessels should then be made. Whilst it is still warm it should be immersed in a bath of water at a temperature of 40° C. Tie a nozzle provided with a stopcock into the vessel previously opened—carotid femoral, or crural, as the case may be—fill the nozzle with saline solution by means of a pipette. Then attach it to a strong brass syringe, previously filled with the injection material, which has been rendered fluid by warming it, and push down the piston so as to drive the injection material into the vessel. This must be done very slowly indeed, and the progress of the injection should be ascertained from time to time by examining the more vascular organs of the body; *e.g.*, the tongue or ear.

The injection of particular organs, such as the kidney, is effected by inserting the nozzle of the syringe into the main artery leading to it; great care and long experience are, however, necessary to obtain successful results.

Instead of the pressure of a syringe worked by the hand the more easily regulated pressure of a column of mercury exerted upon the injection mass contained within a suitable bottle may be substituted, or of air compressed with an air-pump into a bottle communicating with that containing the injection.

The lymphatics can readily be demonstrated in the intestine by injecting them with a watery solution of Berlin blue. This is done by inserting the point of an ordinary hypodermic syringe, filled with the staining solution, into the coats of the intestine in the neighbourhood of a Peyer's patch, and then gently pressing down the piston.

After injection in the case of the entire animal, the body should be transferred to a large vessel, containing ice-cold methylated spirit, without removing the nozzle from the artery. When isolated organs have been injected, they should be suspended in ordinary alcohol in a beaker.

ACCESSORY APPARATUS.

Boxes and Cabinets.—Having prepared and mounted permanent specimens of any tissue, it is necessay to *label* them carefully, noting the method of preparation, the mounting material and date, and to set them aside in a tray box, such as is seen in

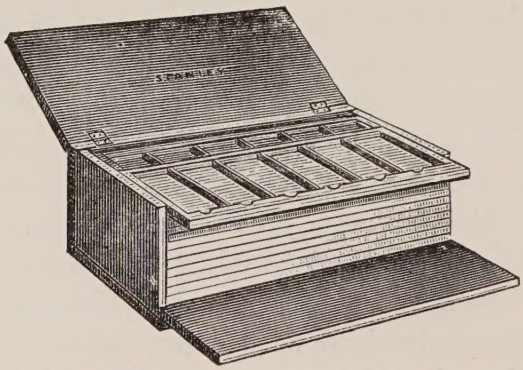

Fig. 17.—Tray Box for Mounted Specimens.

the figure ; these may be obtained of the instrument-makers. The boxes are arranged to hold from one to six dozen slides; when

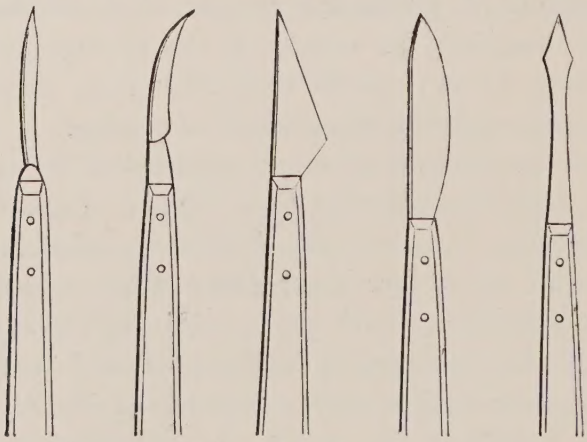

Fig. 18.—Knives for microscopic work.

a larger collection has been made, the worker will find a cabinet very convenient.

Knives.—For the various operations of dissecting tissues,

cutting in pieces and scraping, ordinary scalpels are very useful. For more delicate dissections, etc., other knives are used—*e.g.*, those principally employed by ophthalmic surgeons, and figured above (Fig. 18). It is hardly necessary to add that knives must always be kept very sharp.

Scissors.—Must be fine and sharp, and of several sizes when possible; the diagram below will indicate a useful kind.

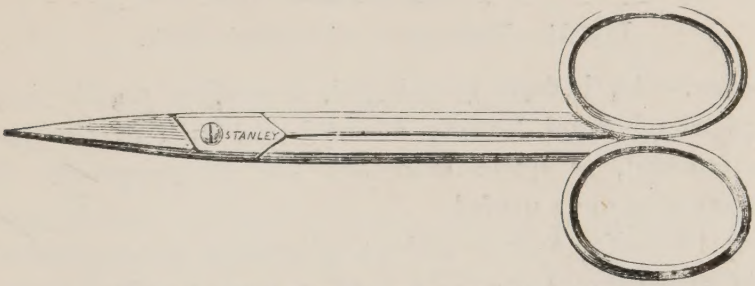

Fig. 19.—Scissors for microscopic work.

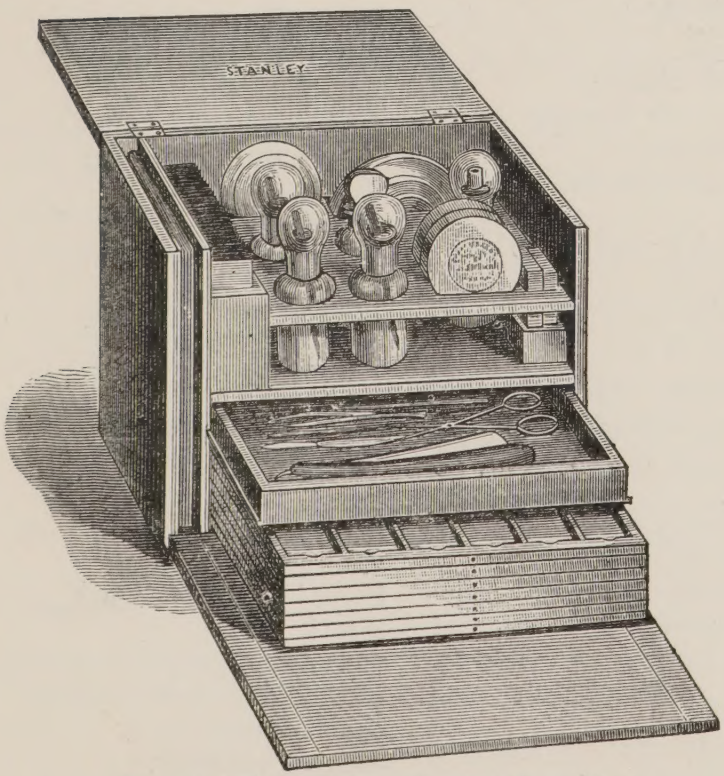

Fig. 20.—Cabinet for microscopic work.

Cabinets for microscopic mounting have been arranged by several instrument-makers containing all the requisites for the

student in a compact compass. The prices vary from £1 1s. to £1 7s. 6d. They save the beginner some trouble.

Forceps.—Should be fine-pointed and slender, if choice be allowed, but dissecting forceps are quite suitable for ordinary work.

Fig. 21.—Forceps for microscopic work.

Needles.—As before mentioned, a sewing needle inserted into a piece of wood, leaving about $\frac{1}{3}$ or $\frac{1}{2}$ inch of the pointed end uncovered, is quite sufficient for all purposes. Broad needles are also very useful.

It may be useful now to add an epitome of the apparatus and reagents which may have to be used in microscopical work.

Apparatus :

Microscope.
A pair of scissors.
A pair of fine forceps.
Two scalpels.
Needles mounted in handles.
Razor.
Glass slides and thin cover-glasses.
Watch-glasses (6).
Glass capsules.
Section-lifters (2).
Labels.
Filter-papers.
A box or cabinet for mounted specimens.
Paraffin or White Wax and Olive Oil mixture.
Gum solution.
Shellac and creasote.

Reagents.—Those in most common use are :

Potassium bichromate, 1 per cent. and 2 per cent. solution.
Ammonium bichromate, 5 per cent. solution.
Ammonium chromate, 5 per cent. solution.
Müller's fluid.
Chromic acid, $\frac{1}{6}$ per cent. solution.
Picric acid.
Osmic acid, gold chloride, etc.
Methylated spirit.
Absolute alcohol.
love oil, or creasote mixture.
Hæmatoxylin solution.
Picrocarmin solution.
Carmine.
Aniline colours.
Eosin.
Sodæ bicarb., 1 per cent., solution.
Acetic acid, „ „
Saline solution, 0·6 per cent.
Tannic and boracic acids.
Glacial acetic acid.
Sodium chloride.
Glycerine.
Dammar varnish.
Canada balsam.
Distilled water.

CHAPTER II.

THE BLOOD.

Human Blood.—Prick the finger with a needle, and apply a clean cover-glass to the drop of blood which issues, so that a little is deposited upon it; then mount on a perfectly clean glass slide, and examine with a $\frac{1}{5}$th or $\frac{1}{6}$th objective. Notice that there are various kinds of corpuscles floating about in a fluid medium.

A. **Coloured Corpuscles.**—These are circular discs $\frac{1}{3000}$ to $\frac{1}{4500}$ of an inch in diameter, and $\frac{1}{10000}$ of an inch in thickness, depressed a little on each side. When seen sideways, biconcave or dumb-bell shaped; of a pale buff colour; but when aggregated, of a reddish tint. They have a tendency to run together, collecting in rolls or rouleaux. Notice a corpuscle as it rolls over, and observe the change in its form, that it is alternately circular and biconcave. The corpuscle has *no nucleus;* the false appearance of a nucleus is occasioned by the refraction of light in passing through a biconcave disc. Prove this by slowly altering the focus. The centre of the corpuscle is seen to become lighter than the periphery when out of focus. The corpuscles consist of two parts: a stroma, which is colourless, and the coloured part, a red crystallizable substance, *hæmoglobin.*

B. **Colourless Corpuscles.**—Their proportion to the coloured varies from 2 to 10 in 1000. When perfectly fresh they are spherical and faintly granular; they quickly alter, and become markedly granular. They are about $\frac{1}{2500}$ inch in diameter *nucleated*, the nucleus not often apparent without the addition of weak acetic acid. They are nearly always isolated; do not

collect together or mix with the coloured discs. They are endowed with the power of spontaneous motion, the so-called *amœboid movement.*

C. Notice that here and there are small more or less rounded, or slightly oval, granules, about one-third the size of the ordinary coloured corpuscles. These are the *blood plates* of Bizzozero.

Action of Reagents.—Make another preparation of a drop of blood on a slide, and add to it a drop of saline solution. Cover it with a cover-glass. Any reagent may now be made to act upon the blood by placing a drop of it on one side of the slip, and applying a piece of filter-paper to the opposite side. A stream of the fluid passes under the cover-glass. This is called **Irrigation.** Irrigate specimens of blood with the reagents mentioned below. The structure of the corpuscles may be thus demonstrated.

(1) *Water:* the coloured corpuscles become smooth and pale, and disappear; their colouring matter being discharged, leaving a colourless stroma.

(2) *Dilute acetic acid:* the same changes take place; the colourless corpuscles swell up, become more distinct, and their nuclei more apparent.

(3) *Tannic acid:* the colouring matter collects in small lumps at the sides of the coloured corpuscles, and is, after a time, discharged.

(4) *Saline solution*, 1 per cent., causes the coloured corpuscles to become crenate and spinous, from shrivelling up of their protoplasm. Syrup and other fluids of considerable density, and slow drying, also produce a similar appearance.

The action of other reagents may also be studied:

(5) *Carbonic acid gas:* a gas-chamber may be made by taking a slide and placing upon it a circle of putty; beneath the putty two small glass tubes are fixed, so that their ends extend into the cells thus formed. The blood is placed upon a cover-glass which is inverted upon the ring of putty. One of the glass tubes is then connected by means of an india-rubber tube with the bottle in which the gas is generated. The stream of gas is allowed to pass into the chamber, and its action upon the blood

corpuscles is observed. The gas passes out of the chamber through the second tube.

The preparation is brought into focus, and *carbonic acid gas* is allowed to pass through the chamber. The coloured blood corpuscles, which had become crenate from the action of the salt solution, again acquire a smooth outline, owing to the swelling up of the parts between the projections. On admitting air to the chamber the corpuscles again become crenate. The nuclei of the colourless corpuscles become more distinct. If frog's blood has been taken instead of human the nuclei of the coloured corpuscles appear more distinct, owing to the coagulation of the substance surrounding the nucleus.

(6) *Chloroform.*—Preparation as for gases. The coloured corpuscles become globular, the hæmoglobin being finally dissolved and discharged into the plasma ; the blood, when seen with the naked eye, being transparent (lake) and no longer opaque.

(7) *Electricity.*—The blood is placed on a slide in such a position that when it is covered it spreads between two poles of tinfoil situated six millimeters apart, which are connected with the secondary coil of an induction apparatus. After a succession of *induction* shocks the coloured corpuscles lose their smooth outline, become crenate, then like mulberries, and finally horse-chestnut-shaped. They swell up, and ultimately become decolourized. The colourless corpuscles swell up and disintegrate, their granules exhibiting molecular movements. With a *constant current* from a single Bunsen's cell the coloured corpuscles at the positive pole undergo changes which correspond to those exhibited under the action of an alkali, and at the negative pole of an acid. The colourless corpuscles assume a spheroidal form, the amœboid movements being resumed as soon as the stimulus has passed.

(8) *Alkalies.*—A mixture of 2 cc. of caustic potash in 1000 cc. of saline solution causes both coloured and colourless blood corpuscles to swell up, burst, and disappear. The coloured corpuscles appear to be more rapidly affected than the colourless.

(9) *Alcohol.*—A mixture of one-third spirit and two-thirds water, acting upon amphibian blood, causes the nucleus to swell and brings into view the nucleolus in the coloured corpuscles.

It also renders the nuclei of the colourless corpuscles more evident, whilst one or more delicate and clear 'blebs' grow from the periphery of the colourless corpuscle. These 'blebs' appear to consist of some colloid substance, into which endosmosis rapidly takes place.

(10) *Carbolic Acid.*—With dilute solutions the coloured corpuscles shrink, and lose their regular contours; after a time they swell up, become pale, and disintegrate; the colourless corpuscles in a dilute solution of carbolic acid in saline solution (1-800 to 1-1600) do not continue their amœboid movements for so long a time or so rapidly as they otherwise would, and the movements are generally of simple extension and retraction of processes, no locomotion taking place. When the corpuscles cease to move, they do not disintegrate as rapidly as when the carbolic acid is absent. With stronger solutions the coloured corpuscles become granular, and the stroma breaks up to form a homogeneous brownish-red material of a high refractive index, which may appear either as an irregular network within the cell, or in the form of globules which tend to coalesce, and are insoluble in water, but are dissolved by carbolic acid. With solutions of $\frac{1}{50}$ to $\frac{1}{20}$, the movements of the colourless corpuscles cease instantly, the cells shrink, and become coarsely granular.

(11) *Of Feeding the Colourless Corpuscles.*—The preparation of blood on the warm stage is irrigated with carmine, vermilion, or aniline blue, in a finely divided state, or with milk. The colourless corpuscles will be found after a short time to have ingested some of the finer particles. The particles are taken into the substance of the corpuscle, by the union around it of two of the protoplasmic processes, and they thus lie at first close to the periphery of the cell, being carried at a later period nearer to its centre.

Of Counting the Blood Corpuscles.—This operation may be accomplished by means of an instrument called the *Hæmacytometer* (Gowers).

It consists of (i.) a metal tray with a central aperture and a pair of clips; the tray is larger than (ii) a glass slip which it holds. The slip is of the ordinary size; it is provided at its centre with a cell which is exactly one-fifth of a millimeter in

depth, and whose floor is ruled in such a way as to form a series of squares, each measuring one-tenth of a millimeter (C). (iii.) A tube (B) with a bore like that of a thermometer, graduated to contain five cubic millimeters. (iv.) A pipette (A) of 995 cubic millimeters capacity, with a fine aperture. (v.) Elastic tubes with mouth-pieces of glass. (vi.) A small glass jar (D). (vii.) A glass stirring rod in the form of a paddle (E). (viii.) A guarded needle (F). (ix.) Cover-glasses, wash-leather, and brushes. (x.) A bottle of sulphate of soda in solution of sp. gr. 1025.

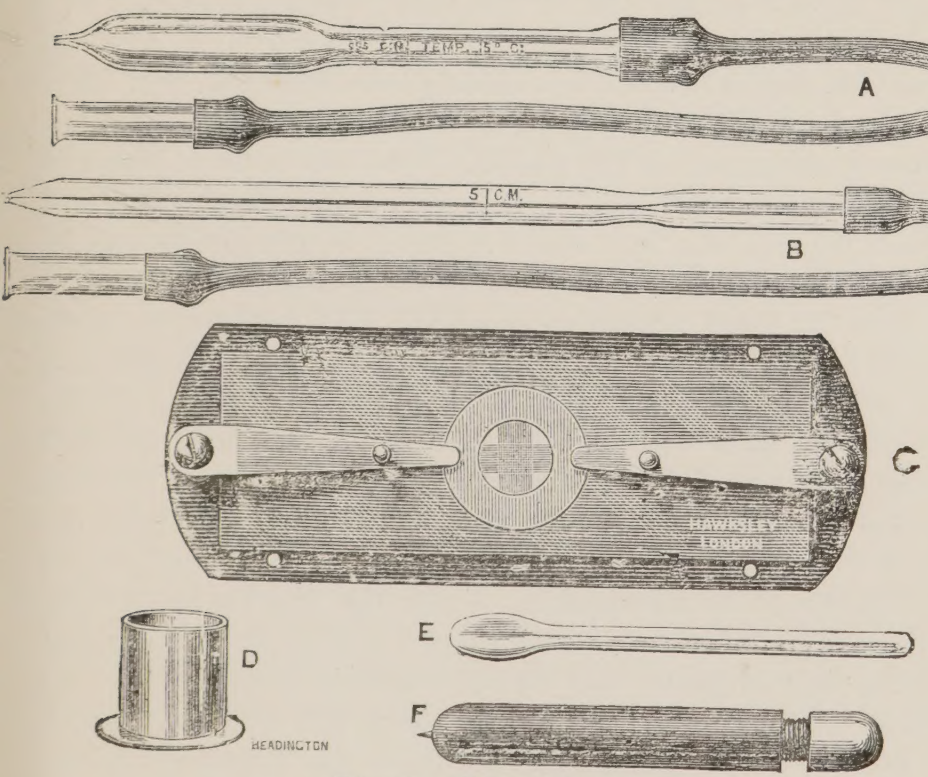

Fig. 22.—Hæmacytometer.

A. Pipette for measuring the saline solution.
B. Capillary tube for measuring the blood.
C. Cell of glass surrounding the place where the divisions are marked on the slide, mounted on a perforated metal slide with side springs to keep down the cover-glass.
D. Jar in which the blood is diluted.
E. Mixer.
F. A guarded needle.

Fix the elastic tubes to the pipette and to the thermometer tube, draw up by suction 995 cmm. of the sulphate of soda solu-

tion, and expel it into the glass jar. With the guarded needle, which should be perfectly clean, draw a large drop of blood from the palmar surface of the last phalanx of the left middle finger. The blood will be more easily obtained, and with less pain, if a handkerchief has been wound tightly round the finger from below upwards ; the blood should be drawn by a single rapid prick of the needle. Suck up the blood into the capillary tube, until it extends slightly beyond the five cubic millimeters mark ; remove the excess by means of a piece of clean blotting paper applied to the end of the tube ; and when exactly five cubic millimeters of blood are left, expel them into the solution of sodium sulphate which has already been measured out. Mix the blood and the sodium sulphate together by a light but rapid rotatory movement of the paddle. Place one or more drops in the cell upon the slide ; cover it with a thin cover-glass, and replace it upon the tray, where it should be allowed to remain for three minutes before the examination is commenced, in order to allow the blood corpuscles to settle. The solution of blood should exactly fill the cell, neither more nor less. In cleaning the cell, it is important that it should not be rubbed, or the micrometer lines will soon be effaced. A stream of distilled water from a wash-bottle, and the subsequent use of the soft camel's hair brush, will be found to be effectual. Examine the blood with a high power ; the corpuscles will be found lying in the squares of the micrometer. Count the number of coloured corpuscles in ten squares, putting down each as they are counted upon a piece of paper ; add up the total and divide by ten ; an average of the corpuscles for each square will thus be obtained. In the case of corpuscles which are upon the boundary lines of the squares, add or omit them according as the centre is or is not on the line bounding the square under notice at the time. In the typically healthy blood of man, each square should contain on the average fifty coloured corpuscles ; in a woman the number is somewhat less. Examine the same ten squares, and count the number of colourless corpuscles ; one or two should alone be present.

Of Preparing Permanent Specimens of Stained Human Blood.—It is found in practice to be anything but easy to pre-

pare specimens of human blood, so that the corpuscles may retain their shape and may be at the same time well stained. After the trial of a large number of different methods, the following is recommended as giving the most satisfactory results. The finger is pricked and a large drop of blood allowed to exude; a perfectly clean cover-glass is lightly drawn upon the top so that a very thin layer of blood adheres, so thin as hardly to be evident until it is dry. It is then dried in the air or put at once without drying into one of the following solutions, viz., chromic acid $\frac{1}{12}$ per cent.; bichromate of potassium $\frac{1}{2}$ per cent.; methylated spirit or absolute alcohol for five or ten minutes, washed in water and again dried. The specimen is now ready for staining. The best dye for this purpose is a *recently prepared* 1 per cent. solution of Spiller's purple in water to which a few drops of alcohol have been added, or a weak spirit solution of rosein. A few drops of one or other dye having been filtered into a watch-glass, the cover-glass is placed upon the surface of the solution blood downwards, and allowed to remain so for from five to ten minutes. It is then removed, washed for some time in a gentle stream of distilled water, dried thoroughly, and mounted in Canada balsam, with or without previous treatment in clove oil for a minute or two. On examination of the specimen the coloured corpuscles should be found of normal shape and coloured purple or red, according to the dye used, whilst the colourless corpuscles are correspondingly stained. The method with Spiller's purple will be found especially useful when blood is examined in diseased conditions in which the existence of micro-organisms is suspected, and is superior to those obtained with many of the aniline dyes (such as methyl-violet).

Amœboid Movements of the Colourless Corpuscles.

In order to demonstrate the amœboid movements of the colourless corpuscles, it is necessary to make use of the *warm stage*. Of this there are various kinds in use. The simplest is a glass slide, to which a perforated circular plate of copper is cemented; this is joined to a projecting rod of the same metal. The rod communicates heat from a spirit-lamp to the plate, upon which

is placed the specimen of blood to be examined. The temperature is regulated by placing a small piece of cacao butter, which melts at 35° C., upon a flattened portion of the rod near the copper disc ; when the butter begins to melt the spirit-lamp should be removed.

The warm stage of Stricker consists of a metal box with central aperture. It is heated either by a copper rod, which can be fixed to it, and which is warmed in the flame of a spirit-lamp, as in the simpler apparatus, or by passing a current of hot-water through the stage itself, or by means of electricity. A thermometer, whose bulb encircles the central aperture, registers the emperature. If hot water be used, an india-rubber tube should be attached to the two brass tubes which project from the sides.

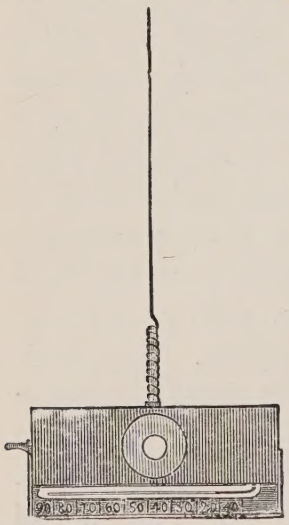

Fig. 23.—Warm Stage.

One of these tubes should then be placed, in connection with a jug of hot water, on a higher level than the stage of the microscope, whilst the other tube acts as a waste pipe. A syphon action may thus be established, and the water will circulate through the stage.

To use the warm stage, place it upon the stage of the microscope, in such a position that the central aperture corresponds with the centre of the largest diaphragm, while the copper rod projects beyond the stage. Place a spirit lamp beneath the rod so that it is heated ; watch the effect of the heat upon the thermometer, and take care that the mercury does not rise above 39°C. When it gets near this point, move the spirit lamp further away. Whilst the stage is being heated, prepare the specimen of blood to be examined. A drop of blood drawn from the finger, or from the tail of the newt, should be diluted with an equal bulk of normal saline solution, and should be received upon a large, square, and perfectly clean cover glass ; a second cover-glass of the same size and shape should also be ready. A little olive oil or melted paraffin should be evenly applied with a camel's hair brush along the edges of the cover-glass holding the blood ; the second cover-glass is carefully put

over it, and the blood spreads out to form a thin uniform layer ; a moist chamber is thus formed by the oil preventing evaporation. The two cover-glasses containing the blood between them are then put over the aperture in the warm stage, and

Fig. 24.—Alterations in the Shape of a Colourless Corpuscle observed for ten minutes.

examined with the highest available power, the movements of a single colourless corpuscle being noted and recorded by drawing it at intervals of a minute (Fig. 24).

Stricker's stage is expensive ; but a less costly modification, which answers the purpose well, is shown in the annexed diagram (Fig. 23). It is provided with a thermometer which accurately registers slight variations of temperature. With the warm stage thus described is combined a gas chamber less rough than the simple form already described.

Gas Chamber.—It may be made by putting the tube leading from the gas generator into connection with either of the tubes which project from each side of the solid projection to which the copper rod is fixed. These tubes are on the side opposite to the thermometer ; they must not be mistaken for the tubes which have been previously described as for the passage of hot water. To use the gas chamber, it is necessary to encircle the aperture with a ring of putty ; the cover-glass containing the preparation to be examined is then placed upon the putty with the tissue downwards, that is to say, in the chamber. The clamp upon the gas tube is then relaxed, and the gas passes into the chamber, when its effect upon the tissue is noted. Air is re-introduced into the chamber by disconnecting the tubing, and sucking through or otherwise passing air into it.

Hæmoglobin Crystals.—These crystals may be prepared as follows : Take a drop of blood from a guinea-pig, rat, or dog, and let it coagulate on a slide ; add a little water, then take up the clot with the forceps, and let several small drops fall from it upon another slide. Cover with a cover-glass. As

these drops evaporate, hæmoglobin crystals of various sizes shoot out from the edges, separately and in bundles, varying in shape according to the animal from which the blood had been taken. In the case of the blood of the three animals mentioned, an easy way to form the crystals is to shake up some drops of their defibrinated blood in a test tube half filled with distilled water ; in the course of an hour or so the sediment will be found to be chiefly composed of hæmoglobin crystals. Ether, or chloroform, also appears to aid the separation of the hæmoglobin from the stroma, and must be used instead of water in the first method of preparation.

Hæmin Crystals consist of the hydrochlorate, or hydrochloride of *hæmatin*—hæmatin is a derivative of hæmoglobin, which easily splits up into *hæmatin* and *globulin*. The crystals of hæmin are thus prepared : A drop of blood is dried on a glass slide. Two or three granules of common salt are added. The blood is powdered and thoroughly mixed with the salt. With a capillary pipette a drop of glacial acetic acid is added, and then the preparation is covered with a cover-glass. The blood and salt should be thoroughly dissolved in the acid. The temperature is gradually raised to the boiling-point over a spirit-lamp, until the greater part of the acid has evaporated. On examination of the residue with the microscope a number of small, reddish-brown rhomboidal crystalline plates are seen. The specimen should be irrigated with distilled water until the excess of salt is washed away, and then dried and mounted in Canada balsam.

Blood Corpuscles of Vertebrates other than Man.

In nearly all *mammalia* the coloured corpuscles are round, disc-like, non-nucleated bodies, similar to those of man, but differing in size. In this respect they vary considerably. In *birds*, *reptiles*, *amphibia*, and *fish*, the corpuscles are oval and nucleated, the nucleus presenting a central elevation on each surface. These corpuscles are larger in birds than in mammalia ; still larger in fish ; and of a yet greater size in amphibia.

The general characters of the colourless corpuscles are similar

in all animals, but are found in much larger proportion in the blood of fish and amphibia than in that of mammalia and birds.

Examination of the Blood of Frogs and Newts.—The blood of newts and frogs affords excellent opportunity of studying the properties of blood corpuscles on an enlarged scale as it were. The coloured corpuscles of the newt are larger than those of a frog, but in each case are *oval* in shape, with a distinct *oval nucleus*, showing a fine intranuclear network; the nucleus is colourless, has a tendency to escape from the body of the cell, but bulges out the central portion of the corpuscle on each side when it is seen sideways. The colourless corpuscles vary greatly in size, the largest not being larger than the coloured corpuscle, and the smallest of the size of its nucleus. Note that some are more granular than others, and some are much more active in their amœboid movements. They possess one or more nuclei. Re-agents act upon amphibian corpuscles much as in the case of human blood. Specimens should be irrigated with (1) dilute acetic acid, (2) tannic acid, (3) boracic acid, 1 per cent., and the effects of the re-agents upon both coloured and colourless corpuscles should be watched. The last-mentioned re-agent causes the colouring matter to collect around the nucleus of the coloured corpuscle, which may then be discharged from the stroma.

To Double-stain the Blood-Corpuscles of Amphibians.—Blood in very thin films should be allowed to dry in the air upon thin cover-glasses. These should then be placed in absolute alcohol for an hour and dried. A few drops of a dilute spirit solution of fuchsin should then be poured upon the dried blood, and should then be at once washed off, and the specimen thoroughly washed with a gentle stream of water from a wash-bottle. The cover-glass should then be dried in the flame of a spirit-lamp and allowed to cool. When ready for the second dye, a small quantity of methylene blue solution should be dropped upon it and allowed to remain for two or three minutes. A second washing with a stream of distilled water should then follow until the washings are all but colourless; and the preparation should finally be dried and mounted in Canada balsam.

CHAPTER III.

EPITHELIUM.

METHODS OF EXAMINATION.—Examples of the various kinds of epithelial cells may be studied either by taking SCRAPINGS FROM THE FRESH TISSUES, or in SECTIONS cut from the hardened material. Where the cells are arranged in layers, or *stratified*, in order to see the relation of one layer to another, the latter method must be adopted ; but the shape and size of the individual cells and their nuclei may be examined according to the first method.

To obtain epithelial cells of various kinds, with their NUCLEI STAINED, it is advisable to subject the tissues from which they are to be prepared to the following process : Immerse in 2 per cent. solution of potassium bichromate for twenty-four hours after removal from the body, and then wash with distilled water until the washings are no longer yellow. Afterwards transfer to a mixture of equal parts of aqueous hæmatoxylin and glycerine for a day, and preserve in glycerine.

A. **Squamous Epithelium.**—(*a*) With a blunt knife, or with the finger nail, scrape off a thin shred from the mucous membrane of the *cheek;* mix it with a drop of normal saline solution on a slide ; place on it a cover-glass, and examine with a one-fifth objective.

The cells are large, flat, roundish, or irregularly polyhedral bodies of various sizes. Their substance is more or less transparent, containing granular matter. The nuclei are small and oval, frequently granular ; they are sometimes missing.

(*b*) Tease a scraping from the inner surface of the *œsophagus* of a cat or dog, prepared after the above method, in a small drop

of glycerine. The nuclei of the cells are seen to be stained with the dye.

B. **Columnar.**—Take a small scraping from the mucous membrane of the *intestine* of some animal (cat, rabbit, or dog), prepared as above, and tease it up with needles in a drop of glycerine on a glass slide.

The cells are cylindrical or conical in form, with a fairly well-defined outline ; protoplasm finely granular ; nucleus clear, oval, well-defined. When an aggregation of cells is seen from above, as on the surface of a villus, it has the appearance of a regular mosaic.

C. **Transitional.**—This type of epithelium is best seen in the *bladder*, from which it may be prepared in manner similar to the above. The shape of the cells should be noted ; some are tailed, others concave on one side, spindle-shaped, or caudate. The nuclei are very large.

D. **Ciliated.**—Scrape lightly the mucous surface of a prepared *trachea*, tease out in glycerine, and examine in a similar manner.

The free border of each cell is provided with cilia, whilst the deeper portion is prolonged into a fine process or tail.

Ciliary Motion.

The movement of cilia may be studied by cutting off with a sharp pair of scissors a small fragment of one of the gills (branchiæ) of a living oyster or mussel, teasing it with needles in a drop of saline solution, mounting and examining in the same fluid. The epithelium scraped from the roof of a frog's mouth, or that obtained from the mucous membrane of the nose, or that lining the alimentary canal of the earthworm, is also well adapted for the demonstration of ciliary motion. The highest available magnification should be used after a general survey has been taken under a low power. Ciliary movement is seen at first to be very rapid ; but it soon becomes slower, and finally ceases.

Effects of Reagents.—Dilute alkalies retard, and then stop the movements. If the cilia are working slowly, or have stopped in a preparation which has just been put up, the careful addition of a very dilute solution of caustic potash or dilute acetic acid,

or the passage over it of carbonic acid, or an electric shock, will generally renew or accelerate the movements for a short time—the ultimate effect, however, being to destroy the cilia.

Carbonic acid first accelerates, then retards, and finally stops the ciliary action, the movements recommencing if air is allowed to take the place of the carbonic acid.

Chloroform retards and finally stops ciliary action; the movements recommencing on the admission of air, if the vapour has not been applied for too long a period.

Warmth accelerates the action of cilia which were previously moving slowly, the movements ceasing at a temperature which is sufficient to destroy the vitality of the cells.

E. **Glandular.**—It is advisable to postpone the study of this form of epithelium until sections of glands, such as the liver or kidney, have been cut and prepared. The cells may, however, be seen by taking a scraping from the freshly-cut surface of a kidney or liver, and preparing it in salt solution.

The cells vary in shape and size, are pale, and fairly well defined.

F. **Pigment** can be studied in scrapings from the *choroid*, *iris*, etc., teased and mounted in glycerine. The cells are either irregular and wide-branching with clear nuclei, or flattened and polygonal.

Endothelium.

Endothelium is a variety of epithelium which, as a single layer, lines serous membranes, blood and lymphatic vessels, etc.

Preparation.—Open the abdomen of a cat, dog, or rabbit which has been killed, a few minutes previously, by bleeding, and carefully remove the *omentum* and portions of the *mesentery*, so that no hair or blood adheres. Place them at once in a solution of silver nitrate ($\frac{1}{4}$ per cent.), gently shaking out the folds in the membrane so that no part escapes the action of the reagent. Allow them to remain in the silver solution for ten minutes and then remove to a vessel of distilled water and well wash them, changing the water after the washing has been completed. Next expose them to sunlight, in the water, until they assume a brownish tint. The tissue is now ready for mounting.

Small pieces should be cut off with a pair of sharp scissors, spread out upon a slide, and mounted in glycerine.

Very excellent specimens of endothelium may also be obtained from the *pleura pericardii* of the same animals, after treatment in a similar way.

In order that not only the outlines of the cells may be traced, but also that their nuclei may be evident, the tissue should be further stained in hæmatoxylin, carmine, or one of the aniline dyes.

A good preparation of endothelium, showing numerous STOMATA, may be obtained from the frog's anterior lymph-sac (*cisterna lymphatica magna*) by filling it with the above-mentioned solution of silver nitrate by means of a capillary pipette, and, after ten minutes, removing the anterior wall, washing in distilled water, and mounting in glycerine.

On examination, the surface of the membrane is found to consist of a single layer of flattened polyhedral cells, variously modified, and forming a mosaic. The nuclei are generally single, and, except in doubly stained specimens, appear as bright and almost colourless oval bodies within the cells. When seen in profile, they occasion projections from the surface.

CHAPTER IV.

THE CONNECTIVE TISSUES.

THESE comprise (1) white fibrous tissue, (2) areolar tissue, (3) elastic tissue, (4) adenoid tissue, (5) gelatinous or embryonal tissue, (6) adipose tissue, (7) cartilage, and (8) bone.

1. **White Fibrous Tissue.**—Typical examples of this tissue are found in *tendons*. Those of the tail of the mouse or rat are exceedingly fine, and may easily be obtained. The tail is cut off close to its base. The skin is removed, and a small piece of the extremity is pinched off between the nails, and is drawn away from the rest of the tail. In separating this piece, a number of fine threads, the tendons, will be noticed. Some of these of moderate size are selected and teased out in saline solution.

Teazing in this manner will show the *fibrous bundles* of which the tendon is made up. Acetic acid may now be added, and the tendons will be seen to swell up and dissolve, leaving their elastic sheaths unaffected.

To demonstrate the *tendon corpuscles* several methods may be adopted. (*a*) The first method is to place the fresh tendons in a mixture of equal parts of aqueous hæmatoxylin and glycerine for two days, tease in glycerine, and press the cover-glass slightly after mounting. (*b*) Another method is to stretch the tendons whilst still perfectly fresh upon a glass slide. The extremities of the tendons are allowed to dry, and by this means they are maintained in an extended condition. A few drops of picro-carmin are placed upon the centre, and are washed away with distilled water after the expiration of half an hour. A drop of glycerine acidified with acetic or formic acid is then added, a

hair is placed by the side of the tendon to obviate pressure, and a cover-glass is put on, the preparation being sealed up in the usual way. (*c*) Thirdly : Good results can also be obtained by mounting the isolated tendons in a 1 per cent. solution of acetic acid, to which one-third its volume of logwood alum solution has been added. The preparation must be examined as soon as possible. (*d*) Fourthly : By treatment with a $\frac{1}{10}$ per cent. solution of osmic acid for an hour, washing in distilled water three hours, and subsequent staining with picrocarmin for twenty-four hours.

To demonstrate the *lymphatic spaces*, the tails of mice, after the removal of the skin, are placed in gold chloride in small pieces, left in the solution an hour, and treated in the usual manner (see p. 32) to reduce the gold. After this has been thoroughly effected they should be placed in a solution of acid (HCl, 2 per cent.) to soften the bone. From this material very thin sections should be cut, stained in hæmatoxylin, and mounted in Canada balsam.

STRUCTURE.—From these various preparations the structure may be made out. The tissue is seen to consist of parallel *bundles of fibres*, which vary in thickness and are held together by a homogeneous and albuminous cement substance. The individual fibres forming the bundles are straight or wavy, and are extremely delicate. The acetic acid added to the tendons causes *the fibres to swell up* and to disappear, owing to the presence in the tissue of a substance which is readily convertible into gelatine. The bundles of fibrils are surrounded by a more or less complete sheath of elastic tissue, which is not acted upon by dilute acids ; hence the constricted appearance seen in the tendons to which acetic acid has been added.

On examination of the tendons treated to show the corpuscles, they will be found to consist of parallel bundles of fibres, whose substance is almost colourless, arranged in groups. Between each two groups is a lymph-channel, in which lie nearly parallel layers of delicate stained cells—*the connective-tissue* or *tendon cells* —forming for each channel a single continuous row of irregular angular plates. Each plate is provided with a more deeply staining nucleus. The cells are separated from each other by a cementing substance, and they possess fine processes. Each cell

presents a straight ridge—*the elastic stripe*. This ridge is formed by the union of two or three concave portions of which the cell is composed, to enable it to adapt itself to the curved surfaces of the tendon bundles. *The lymphatics* are demonstrated in transverse sections of the tail, stained in chloride of gold ; dark masses are seen in the tendon, corresponding to the *lymphatic channels*, filled with an albuminous fluid plasma. Radiating from these masses are fine septa—*the cement substance* —binding together the contiguous bundles.

2. **Areolar Tissue.**—This form of tissue is really a variety of fibrous tissue, and is best seen in specimens of intestine and skin. By the injection into the subcutaneous tissue of a rat which has just been killed of a 0·2 per cent. solution of nitrate of silver or osmic acid, a small artificial bulla is formed. This is allowed to remain for ten to thirty minutes, and is then opened with a pair of fine curved scissors, and the delicate subcutaneous tissue is rapidly removed and spread out on a glass slide. It is immediately covered with a thin glass, and the preparation is stained for twenty-four hours with picrocarmin. Glycerine is passed through until all the superfluous staining material is removed, after which the preparation is sealed up.

STRUCTURE.—The tissue is composed of delicate bundles of *ordinary white fibrous tissue*, some of the fibres are fibrillated, and all interlace with each other ; the meshwork thus formed contains a few very fine fibrils of *elastic tissue*, which become still more evident if dilute acetic acid have been allowed to act upon the preparation. The interspaces are filled with lymph, containing *lymph corpuscles*. Large *plate-like cells*, which appear to lie upon the surface of the bundles of fibres, are also seen. When viewed sideways, these cells have a branched appearance, and form the *plasmatic cells*. *Fat cells* are also present.

3. **Elastic Tissue.**—The coarse and larger fibres may be demonstrated by teasing out a small piece of the ligamentum nuchæ of an ox in glycerine, and the finer fibres by mounting a piece of the omentum of a rabbit or cat on a slide, irrigating it freely with dilute acetic acid, and then staining with hæmatoxylin, or with an aniline dye, preferably a magenta or fuchsine, and preserving it in glycerine.

STRUCTURE.—On examination elastic fibres are either *thick*, well-defined, and form bundles; or they are *fine*, shining, and not in bundles. They *branch* dichotomously, and *anastomose* with each other to form a real network; when torn, they *curl up* at the ends. They do not swell up when treated with acids, and on prolonged boiling they yield elastin and not gelatine as do white fibres.

4. **Adenoid Tissue**, retiform or lymphoid tissue, can be demonstrated by cutting and staining sections of a lymphatic gland which has been kept in dilute spirit. After staining with hæmatoxylin the sections should be shaken in a test tube half filled with water for an hour or more. The portions of the broken-up sections should then be dehydrated, cleared, and mounted in Canada balsam.

Adenoid tissue forms the basis of the spleen, the lymphatic glands, the tonsils, thymus, Peyer's glands, etc.

STRUCTURE.—It consists of a fine reticulum composed of the branchings of corpuscles, which either retain or do not retain their nuclei. In the reticular spaces are contained, closely packed together, very small corpuscles with nuclei almost entirely filling them—the *lymphoid corpuscles*. In order to study the reticulum the corpuscles must be removed, and this is the object of subjecting the sections to the treatment by shaking. Chemically, the fibres of the reticulum differ from both yellow elastic and white fibrous tissue.

5. **Gelatinous, Embryonal, Mucous, or Wharton's Tissue** is present in the umbilical cord and in the fœtal skin. It is obtained from a bulla formed by the injection of a dilute solution of gold chloride, in a stronger solution of which it is subsequently stained.

STRUCTURE.—In the youngest condition, it will be found to consist of a transparent jelly-like substance containing a hyaline mucous substance within a reticular framework. At a later period *bundles of fibrous connective tissue* are apparent, as well as *branched cells*, *blood-vessels*, and *fat cells* in an early stage of development. The tissue yields mucin on boiling. *The vitreous humour* appears to be a variety of this tissue in which the branched cells have lost their processes.

6. **Adipose Tissue** may be seen best in sections of scalp, or in pieces of omentum stained in hæmatoxylin. A small piece of fat, which has been partially teased, may be placed in ether for twenty-four hours, and the fatty portion will be dissolved out. Examine also the preparation of areolar tissue formed by the injection of nitrate of silver as well as sections of fatty tissue stained with osmic acid in order that the action of that reagent upon the fat-cells may be observed.

STRUCTURE.—Adipose tissue consists of a matrix or network of areolar tissue containing fat-cells. *Fat-cells* are clear, well-defined, rounded vesicles of varying size, filled with an oily fluid, which often gives rise after death to *crystalline needles*, radiating from the centre of the cell. In successful preparations, especially of the omentum, a fine zone of *protoplasm*, with a *nucleus* at one pole, can be seen surrounding the cell more or less completely. The fat-cells may either form compact masses, with only a small amount of connective tissue, or they may be more or less isolated. The tissue possesses a capillary network of *blood-vessels*. Between the fat-cells, flattened nucleated *connective-tissue cells* may be demonstrated.

7. CARTILAGE.

Cartilage consists of two parts : (1) Cells ; and (2) Matrix, or intercellular material. According to the nature of the matrix, cartilage is classed as :

a. Hyaline cartilage.
b. Fibro-cartilage.
c. Elastic cartilage.

a. **Hyaline Cartilage** is found in various localities, from which the names costal, tracheal, articular, ossifying or intermediary, and embryonal, are derived. Portions of each of these cartilages should therefore be examined. The cartilages may be prepared in a solution of chromic acid 1 in 600, in a saturated solution of picric acid, or by the gold method. In every case the sections, which must be very thin, should be stained with carmine or hæmatoxylin. It may be as well, also, to stain some portion of the nasal cartilages in osmic acid.

STRUCTURE.—All cartilage, with the exception of the free ex-

tremity of articular cartilage, possesses a delicate vascular connective-tissue sheath—*the perichondrium*. *The cartilage-cells* are spherical or oval protoplasmic bodies, generally containing a single nucleus. The cell protoplasm forms a fibrillar meshwork which is contracted in embryonal and articular cartilages. Each cell is placed in a lacuna, enclosed by a firm, structureless, but transparent *matrix*, yielding chondrin on prolonged boiling. In growing cartilage, a special layer—*the limiting membrane*—can be distinguished between the lacuna and the ground substance. In some cases a single lacuna may contain more than one cell, due to reproduction of the cartilage-cell by fission ; and the various stages in the division of cells may often be well seen. Near the perichondrium the cartilage-cells become flattened and smaller ; near the articular surface they are branched ; in ossifying cartilage they are arranged in parallel rows. The matrix possesses an anastomosing system of lacunæ and canals in connection with the *lymphatic* system.

b. **Fibro-Cartilage.**—White fibro-cartilage occurs in the intervertebral substance and in sesamoid bones, and may be well seen in longitudinal sections of a mouse's tail stained in gold chloride.

Structure.—It consists of groups of slightly flattened *cells*, each with a round nucleus, and enclosed in a distinct capsule. The matrix is composed of bundles of fibrous tissue, which sometimes form lamellæ with occasionally a concentric arrangement.

c. **Elastic Cartilage.**—This form of cartilage occurs in the lobe of the ear, in the epiglottis, in the cornicula laryngis, and in the Eustachian tube. Sections should be cut of the pig's ear, in which it is seen very well, stained in hæmatoxylin and mounted in Canada balsam.

Structure.—Elastic cartilage in the adult is hyaline cartilage permeated by elastic fibrils. *The fibrils* are arranged so as to form the trabeculæ of a reticular framework ; they branch and anastomose very frequently. The meshes contain fusiform groups of large nucleated *cells*, surrounded by a larger or smaller amount of hyaline cartilage substance.

Ossifying or Calcifying Cartilage.—In order to show ossifying cartilage, a fœtal femur or other long bone is decalcified with the chromic acid and hydrochloric acid mixture, and sec-

tions both transverse of the shaft and longitudinal of the extremities should be cut. The sections may be doubly stained in picrocarmin (or eosin) and hæmatoxylin. This variety of cartilage is found at the junctions of cartilage with spongy bone in the epiphyses and ends of the shafts of long bones, and represents the method by which bone increases in length. It will be seen that the stages of the formation of bone in this way correspond almost exactly with the so-called development of bone in cartilage (p. 70). In a longitudinal section through the end of a growing long bone the following layers can be seen at the junctions of the cartilage and bone :

(1) Ordinary hyaline cartilage covered with perichondrium in the diaphysis of a long bone at the junction of the cartilage with the spongy bone, a characteristic arrangement of the cartilage cells in longitudinal columns. The cells are also seen to be conical in shape, pressed together and flattened transversely.

(2) A transparent layer, in which the lacunæ of the cartilage cells are seen to be enlarged, the matrix diminished, the cells enlarged and transparent, and their nuclei swollen.

(3) The lacunæ are becoming confluent, and the matrix calcified.

(4) The enlarged lacunæ are seen to be filled with marrow, and the trabeculæ of calcified cartilage are covered with layers of marrow-cells, amongst which are enlarged multi-nucleated mother-cells (giant-cells).

(5) The marrow-cells (osteoblasts) are seen to have deposited layers of low ossific material upon the calcified trabeculæ, and at the same time the calcified centres have become less free from absorption.

(6) The calcified centres of the trabeculæ have disappeared, leaving ossific trabeculæ which form the spongy bone.

8. BONE.

The fresh bones of any small animal, well cleared of the surrounding tissues, should be placed for two or three weeks in a large quantity of $\frac{1}{2}$ per cent. solution of chromic acid, containing five drops of hydrochloric or nitric acid to each ounce of the solution. When the whole of the earthy matter is

dissolved out, sections should be cut with a razor in various directions, and examined in glycerine.

As the preparation of specimens of hard bone requires much time, the student is advised to buy both transverse and longitudinal sections of that material.

The structure can be best made out in a specimen of hard bone.

a. **Compact Bone.**—In transverse sections of the compact tissue of long bones are seen Haversian systems, more or less perfect, and Haversian interspaces. Each system consists of the central *Haversian canal* (which is generally round or oval, with an average diameter of $\frac{1}{500}$ in., and is lined with a delicate membrane continuous with the periosteum), surrounded by concentric lamellæ of bone, between and in which are the lacunæ and canaliculi. *Lacunæ* $\frac{1}{2000}$ in. in length, generally well-marked, contain shrunken bone corpuscles. *Canaliculi* usually indistinct, but, when seen plainly, forming a complete system of communication between the lacunæ of the same and neighbouring Haversian systems and interspaces. They contain in the fresh condition prolongations from the bone corpuscles. Each Haversian system is more or less isolated from its neighbour by a layer of bone which contains but few canaliculi. The *Haversian interspaces* are the portions of bone filling up the interval between one or more of the circular Haversian systems. They do not contain any central canal; their general characters are otherwise similar to the systems.

In longitudinal sections the Haversian canals which run longitudinally are seen to anastomose freely by transverse or oblique channels. The lacunæ and canaliculi present much the same characters as in transverse sections. The Haversian canals which run near the circumference of the bone may open on the outer surface so as to admit blood-vessels from the periosteum, whilst those opening into the medullary canal receive blood-vessels, and in the case of the larger ones, medulla from the interior of the bone.

In preparations of calcified bone it may be seen that the lamellæ are bolted together by *the perforating fibres of Sharpey.* Bone situated immediately beneath an articular cartilage differs

in not possessing Haversian canals, in the lacunæ being larger than in ordinary bone, and in being destitute of canaliculi. *The periosteum* covering the free surface of bones consists of an external layer of dense fibrous tissue supplied by capillary blood-vessels, and of an internal osteogenetic layer containing a plexus of delicate connective-tissue fibrils ; in the meshwork formed by these fibrils are capillary blood-vessels and a number of nucleated cells. *The medulla* is of the yellow kind, and is chiefly composed of fat-cells, with intervening membranes of flattened connective-tissue cells ; it also contains numerous cells possessing one or two nuclei.

b. **Spongy or Cancellous Bones.**—In spongy bones, *e.g.*, a vertebral, carpal, or tarsal bone, the tissue consists of bone trabeculæ, forming a more or less open framework, in which is embedded the medullary substance. Bone trabeculæ contain lacunæ with bone corpuscles and ill-developed canaliculi. The medullary substance is of the red kind ; it is rich in blood-vessels, and in cells having the characters of lymph corpuscles, fat-cells, etc.

The Development of Bone.—It is usual to describe two ways in which bone is developed, viz., (1) in cartilage, (2) in membrane ; but in reality the shaft of all permanent bones is developed in membrane, as will be seen in the following description. The so-called development in cartilage is really a preliminary stage, and all bone which is so formed, except at the growing ends, does not remain a part of the permanent bone, but it is reabsorbed nearly as soon as it is made.

In the account of the formation of bone in cartilage, the stages under which the process is described are more or less arbitrary, being inserted for the convenience of description.

In Cartilage.—This must be studied both in longitudinal and transverse sections of fœtal bones in various stages of development, doubly stained in the manner above described. Stage i. *Hyaline cartilage* covered by perichondrium. *The perichondrium* consists of an outer layer of embryonal connective tissue, and an inner osteogenetic layer containing spherical cells—the future osteoblasts and blood-vessels. Stage ii. *The inner layer of perichondrium penetrates the cartilage*, forming for itself channels by

absorption, and carrying with it blood-vessels and cells. The growth of the perichondrium inward starts at the centres of ossification. Stage iii. *The primary marrow cavities* are formed by the appearance of lacunæ near the cartilage channels, which then become confluent, whilst the trabeculæ separating neighbouring lacunæ become calcified. The primary marrow filling the marrow cavities is the periosteal ingrowth containing the vessels and cells. Stage iv. *The calcified trabeculæ become ensheathed with osseous material*, and are then absorbed. A network of osseous trabeculæ instead of a network of calcified cartilage is thus formed, whilst the whole tissue resembles spongy bone (endochondral bone). The surfaces of the osseous trabeculæ are covered with osteoblasts, whilst the cavities separated by the trabeculæ are filled with marrow rich in vessels and cells. So far we have almost the same stages as described above, under the head of ossifying cartilage. Stage v. The endochondral spongy bone is absorbed, from the centre outwards ; the large *medullary cavity* is thus formed. *Bone from the periosteum* is also simultaneously developed round the endochondral bone. *The osteoblasts* multiply and become converted into the osseous matrix and into bone corpuscles. The meshes of the spongy periosteal bone are *the Haversian spaces ;* they contain marrow from which a series of concentric lamellæ are formed. The spaces are thus gradually reduced to *Haversian canals.* Stage vi. *All the endochondral bone is absorbed*, and the ossified trabeculæ are represented by the interstitial substance separating the concentric Haversian lamellæ.

As the bone increases in thickness, though continued by the formation of new bone on the outside by the periosteum, the primary periosteal bone is pushed towards the centre and is absorbed, first of all becoming more spongy in its nature ; the bone formed later from the periosteum being much denser and more compact in structure than that first formed.

In Membrane.—This is best studied in stained and prepared sections of fœtal lower jaw. The membrane corresponds to the future periosteum ; it consists of two parts, as above. Stage i. The cells of the osteo-genetic layer—*the osteoblasts*—increase and form the osseous matrix by excreting ossein around them-

selves, thus forming ossified trabeculæ which start from the centres of ossification. Stage ii. Portions of the trabeculæ are absorbed, whilst, as in endochondral bone, concentric lamellæ are formed by the marrow in the Haversian canals.

The formation of intramembranous bone is identical with the formation of periosteal bone. The absorption of osseous substance is in nearly every case associated with the presence of multinucleated giant cells—*the osteoclasts.*

CHAPTER V.

MUSCLE.

THERE are *two varieties* of muscular tissue, (1) striped and (2) unstriped, or plain. Of the striped muscle, that which is found in the heart differs somewhat from the rest.

In order to study the structure of muscular tissue the following preparations should be made :

(*a*) *Of Fresh Muscle.*—(i.) With a couple of needles open the chitinous shell of the leg of a water-beetle or of a cockroach, turn out the muscle on a slide tease and mount in saline solution ; edge round the cover-glass with dammar, or paraffin, to avoid evaporation, and examine at once. The striations are very marked and coarse. If examined whilst the muscle is still irritable it may be that the wave of contraction will be seen in the fibre. (ii.) Tease a piece of fresh muscle from the crab, or from the thin muscles attached to the jaw of the frog.

(iii.) To see *the sarcolemma*, treat fresh muscle with dilute acetic acid.

(iv.) To see *Cohnheim's fields*, make sections of frozen fresh muscle and examine in salt solution.

(v.) To see the *nerve terminations*, treat muscles from the frog or lizard, about five hours after death, according to the chloride of gold method.

(vi.) To see muscle *terminations in tendon*, take a thin, straight muscle, ending in a tendon, from a frog, put in a ·5 osmic acid solution for several hours, and tease in glycerine—and macerate another in caustic potash, 40 per cent. solution.

(vii.) To see *transverse muscle discs*, immerse in 1 per cent. acetic acid or 0·1 hydrochloric acid, and tease in glycerine.

(*b*) *Of Hardened Muscle.*—Voluntary and heart muscles from various animals should be hardened in chromic acid and spirit; pieces should be taken and teased in glycerine, with or without previous staining in hæmatoxylin.

To see the *primitive fibrillæ*, macerate the muscles of the lower jaw of a frog, or the tail of a tadpole, in a saturated solution of picric acid for a week, or in 5 per cent. chromic acid for a few days, and tease in glycerine.

To see the general *relations of muscle fibres, the position of the nuclei and the appearance of sections of muscle cut in different directions*, thin sections should be made (*a*) of the *tongue of a rabbit*, and (*b*) of the *heart of a rabbit*, or guinea-pig, which should be stained in hæmatoxylin, prepared, and mounted in Canada balsam.

(*c*) *Of Unstriped Muscle.*—Unstriped tissue may be demonstrated by distending a piece of rabbit's intestine with saline solution, leaving it in a 1 per cent. solution of anilin black for twenty-four hours, and stripping off the outer coat with forceps. Small pieces may be mounted in glycerine.

The muscular-fibre cells may also be seen by stripping off pieces of the outer coat of the intestine, which has been macerated in a solution of chromic acid or potassium bichromate; they may be washed, stained, and teased in glycerine.

The tissue is well seen in sections of the stomach of the newt or frog which have been hardened in chromic acid and stained in hæmatoxylin; very large individual cells are well shown in the mesentery of the newt, which has been put fresh into ammonium bichromate 5 per cent. for twenty-four hours, stained in hæmatoxylin and mounted in glycerine.

With the aid of the preparations indicated above, the general **structure** *of the muscular tissue* can be made out. (1) It will be as well to consider, first of all, the *striped muscle* of the *voluntary* or skeletal type. It consists of long *fibres*, which are cylindrical, but appear in transverse section as rounded polygons. Each fibre is made up of a number of exceedingly fine and delicate

filaments, *the fibrillæ*, enclosed within the sarcolemma. The fibres are aggregated into *bundles;* several bundles forming *fasciculi*, and these the anatomical *muscle*. *Perimysium* or fibrous connective-tissue surrounds the bundles; from it pass off between the muscle fibres small processes of connective-tissue, with cell plates and plasma cells—*the endomysium*.

So far, with the exception of the fibres and of the endomysium, the appearances may be made out with the naked eye, or with a very low power of the microscope. But now the higher powers are required.

Each fibre will be seen to consist of broad dim bands of highly refractive substance, representing the contractile portion of the muscle-fibre—*the contractile discs*—alternating with narrow bright bands of a less refractive substance—the *interstitial discs*. After hardening, each contractile disc becomes longitudinally striated, the thin oblong rods thus formed being the *sarcous elements* of Bowman. The sarcous elements are not the optical units, since each consists of minute doubly refracting elements —*the disdiaclasts* of Brücke. When seen in transverse section, a muscular fibre appears to be subdivided by clear lines into polygonal areas—*Cohnheim's fields*, each corresponding to several sarcous-element prisms. The clear lines are due to a transparent interstitial fluid substance pressed out of the sarcous elements when they coagulate. *The sarcolemma* is a transparent structureless elastic sheath of great resistance, which surrounds each fibre. From the sarcolemma, transverse membranous septa—*the membranes of Krause*—extend inwards across the muscle at regular intervals. By these septa the muscle-fibre is divided into equal-sized *muscle compartments*, each containing one contractile disc. The membranes of Krause are so placed that each passes across the middle of an interstitial disc, which is thus divided into two *lateral discs*.

A thin transverse median disc—*the disc of Hensen*—is occasionally seen to divide the contractile disc. In some fibres, chiefly those from insects, each lateral disc contains a row of bright granules forming *the granular layer* of Flögel. The fibres contain nuclei, which are roundish, ovoid, or spindle-shaped in different animals. These nuclei are situated close to the sarco-

lemma, their long axes being parallel to the fibres which contain them. Each nucleus is composed of a uniform network of fibrils, and is embedded in a thin more or less branched film of protoplasm. The nucleus and protoplasm together form the muscle-cell or *muscle corpuscle* of Max Schultze.

In injected specimens it will be seen that the arteries and veins are in the perimysium ; that the capillaries are in the endomysium, between the fibrillæ ; and that the capillary plexus is elongated. The individual capillaries run parallel with the muscle-fibres, and anastomose with each other by short branches.

Heart Muscle.—The fibres seem to be very small and finely striated. The striæ are generally indistinct, often showing only as coarse granules. There is no sarcolemma. Many of the fibres anastomose and branch. The nuclei are in the centre of the fibres.

Unstriped Muscle. — Is made up of bundles of cells, bound together by an albuminous cementing substance — the *endomysium*—in which lie connective-tissue cells and a few fibres. The *perimysium* continuous with the endomysium is the fibrous connective-tissue surrounding and separating the bundles of muscle-cells. Fibres, fusiform, band-like, or spindle-shaped, containing elongated or staff-shaped nuclei, are placed midway in the fibres. The ends may be split into two or more parts, as may frequently be seen in the cells obtained from arteries and veins. Each *muscle-cell* consists of a fine sheath, probably elastic ; of a central bundle of fibrils representing the contractile substance ; and of an oblong nucleus, which includes within a membrane a fine network anastomosing at the poles of the nucleus with the contractile fibrils. The ends of the fibres, which are usually single, may sometimes be divided. There is no sarcolemma.

CHAPTER VI.

NERVOUS TISSUE.

NERVOUS tissue consists of *nerve-fibres* and *nerve-cells*, together with *supporting connective-tissue*. Nerve-fibres should be studied first of all in the trunks of cerebro-spinal and sympathetic nerves, and in the olfactory, optic, and auditory nerves. Afterwards nerve-fibres, as well as nerve-cells, should be examined in the central nervous system—in the spinal cord and brain.

NERVE FIBRES.

Preparation.—A medium-sized nerve-trunk, *e.g.*, the sciatic nerve of a rabbit, guinea-pig, or frog, should be removed from the recently-killed animal, and cut into short lengths. One or more of these lengths should then be placed in the following solutions: (*a*) *Osmic acid*, 1 per cent., (*b*) *Gold chloride*, ·5 per cent., (*c*) *Silver nitrate*, ·25 per cent., (*d*) *Hæmatoxylin* or *carmine*, and teased with needles whilst in the reagent in order that it should penetrate properly.

The osmic acid should be allowed to act for an hour or two, and the fibres, when teased, will be found to show the medullary sheath, the nodes of Ranvier and the sections of Lantermann—the first being stained black.

The gold chloride method will demonstrate the external nerve sheath and the axis cylinders.

The silver nitrate requires about a quarter of an hour for its action; it will show the endothelium covering the nerve-bundles, and also, by more prolonged action, the nodes of Ranvier.

The hæmatoxylin and carmine will show, in a good prepara-

tion, the nucleated external nerve sheath and the axis cylinder. A considerable time is necessary, however, to accomplish this action—twenty-four hours or more—and very frequently the stain will not penetrate to the axis cylinders even then, unless the fibres have been placed first of all in absolute alcohol, ether, or chloroform.

The fibres should in all cases be teased and mounted in glycerine.

Nerve-trunks of a rather larger size should be pinned out straight on a piece of cork, and hardened in chromic acid and spirit solution. Of these transverse sections should be cut, stained in hæmatoxylin, prepared and mounted in Canada balsam.

The auditory, sympathetic, and optic nerves may also be examined by teasing, and in transverse section after hardening and staining.

Structure.—Nerve-fibres are of two kinds, (*a*) *Medullated*, or (*b*) *Non-medullated*. The former chiefly make up the cerebro-spinal, and the latter the sympathetic system.

(*a*) The cerebro-spinal nerve-trunks, with two or three exceptions, are composed of a variable number of bundles of nerve-fibres (*funiculi*), each of which has a special sheath (*perineurium* or *neurilemma*). These bundles are inclosed in a firm fibrous sheath (*epineurium*), which also sends in processes of connective-tissue supporting and connecting the bundles together. Within the funiculi, between the fibres, is a delicate supporting tissue (the *endoneurium*). Each medullated nerve-fibre is made up of the following parts : (1) Primitive nerve sheath, or nucleated sheath of Schwann ; (2) Medullary sheath, or white substance of Schwann ; (3) Axis cylinder, primitive band, axis band, or axial fibres.

(1) *The nucleated sheath of Schwann* is the external layer of the fibre. It appears to be a simple, transparent, colourless, homogeneous structure, containing a varied number of oval nuclei embedded in protoplasm.

(2) *The medullary sheath*, or the intermediate layer, forms the greater part of the nerve-tube. It surrounds the axis cylinder, and has a double contour ; and at regular intervals are constric-

tions in the medullary sheath (*Ranvier's constrictions*), caused by the thinning or interruption of the medullary substance. In it may be demonstrated, especially by the action of osmic acid, the *sections of Lantermann*, of which its structure is said to be made up.

(3) *The axis cylinder* is situated in the centre of the nerve-tube, and appears as a faintly-marked band, with an exceedingly fine and even outline. It is made up in the prepared specimen of a number of most delicate fibrils (*primitive fibrils*).

Between the axis cylinder and the medullary sheath there is said to be a small space containing, in the recent state, albuminous fluid. At the constrictions of Ranvier, in nerves stained with silver nitrate, a black cross is seen, due to the action of the silver on the albuminous cement substance between the constricting folds, and to its penetrating and staining the cement substance surrounding the axis cylinder for a variable distance.

(*b*) In the optic and auditory nerves the nerve trunk is not made up of distinct bundles, and consequently there is no perineurium, but the trunk may be looked upon as a single bundle of fibres, and if so, the epineurium must be said to be absent. The nerve-fibres in the trunk have no external nerve sheath, but, instead, the endoneurium, which may also be called *neuroglia*, is more marked than in the other cerebro-spinal nerves.

(*c*) In the sympathetic system, as well as in the olfactory nerve, the nerve fibres possess no medullary sheath, and so are called non-medullated nerve-fibres.

SPINAL CORD.

Preparation.—Short lengths (about $\frac{1}{8}$ to $\frac{1}{4}$ inch) of the spinal cord of the cervical, dorsal, and lumbar regions of a calf, sheep, or pig, should be hardened in a 5 per cent. solution of bichromate of ammonium or the usual mixture of chromic acid and spirit for a week, and should then be transferred to spirit.

A solution of eosin is recommended for staining the sections, and carmine or picrocarmin and hæmatoxylin act well. Stephens' blue-black ink diluted one half or two thirds, or anilin black, 1 per cent. solution, stains the ganglion cells excellently. Double staining with eosin and hæmatoxylin produces good results.

In order to study the areas or tracts in the spinal cord it is necessary to obtain pathological material from the human subject in which tracts of degeneration may be demonstrated. The following method is recommended for the purpose. It is called the Weigert* hæmatoxylin method.

Solutions required are :

1.	Hæmatoxylin ext.	1 grm.
	Absolute alcohol	10 cc.
	Distilled water.....	90 cc.
2.	Ferricyanide of potass.	$2\frac{1}{2}$ grm.
	Borax.....	2 grm.
	Water.....	100 cc.

Grind the hæmatoxylin extract in a mortar with a little alcohol, then add the alcohol and water. The solution is better if kept a day or two before using.

Method of Using.—The sections of brain or cord are placed in solution 1, till they are stained a deep and uniform black. This may take any time from one hour to twenty-four. The operation of the stain is much helped by keeping the preparation in an incubator at a temperature of 40° C. Overstaining is scarcely possible. The sections are then taken out, slightly washed in clean water, and placed in solution 2. A slight brown cloud is then seen to come from them, and the colour gradually disappears from the grey matter, or from areas of degeneration if there be any. This operation is complete when no more brown colour appears in the yellow fluid. The *medullated nerve-fibres* will now be found to be deeply stained a rich purple, all the rest of the section being a brownish-yellow.

Thus the brain or cord may be stained *in toto* by this method, but the operation, of course, takes much longer. An average time, for instance, for the penetration of the stain into a slice of cord or medulla $\frac{1}{16}$ inch thick would be three or four days, and about the same time would be required for the developing process in solution 2.

Precautions.—The material should be hardened in Müller's fluid, or bichromate of potash. If bichromate of ammonium or chromic acid have been used it should be *entirely* removed by

* *Weigert*, Fortschritte der Medicin, 1884, p. 190. We are indebted to Dr. Tooth for this account. He has used the method with much success.

constant washing. The best results are obtained from material from which every trace of the hardening fluid, whatever it may be, except alcohol, is removed. If the material has been penetrated with wax or paraffin for the purpose of section cutting, it will never take the stain satisfactorily or with certainty, however well the wax may have been removed.

Tracts of Degeneration.—By means of disease various tracts are mapped out in the cord, showing in many cases the courses of the fibres. These tracts, as seen on transverse section, are as follows : In the postero-lateral column, a little nearer the grey matter than the exterior, a somewhat oval or wedge-shaped area, consisting of fibres which have decussated in the anterior pyramid of the medulla and passed over to the lateral region. This area forms the *crossed pyramidal tract*, which diminishes in size from above downwards, and it can be traced as low as the third or fourth sacral nerve. Outside this a very narrow area—the *direct cerebellar tract*—can be traced down as far as the second lumbar. On either side of the anterior fissure the uncrossed, or *direct anterior pyramidal tract*, diminishes downwards until it disappears about the middle of the dorsal region. On either side of the posterior fissure is the *posterior median column, or column of Goll*, traceable as low down as the middle of the dorsal region ; outside this, the *postero-external column, or column of Burdach*, can be distinguished. Between the anterior cornu and the exterior of the cord anterior to the direct cerebellar tract is *Gower's tract of* ascending degeneration.

In Structure the spinal cord consists of (1) *white matter*, (2) *grey matter*, and (3) *neuroglia*. Observe the structure of the spinal cord by means of a transverse section taken from the region of the cervical enlargement. In the first place examine the stained section mounted in Canada balsam under a two-inch objective, or else with a hand magnifying-glass. Notice the roughly oval shape of the section.

The central and more deeply-stained portion is the grey matter. It is less granular than the white matter seen round the circumference. It offers for examination two crescentic masses with their concavities turned outwards, joined across the middle by a transverse portion (the grey commissure). In the

centre is a small canal lined by a layer of columnar cells, which in young animals are ciliated. Each crescent presents (*a*) *the anterior horn*, or cornu, which is short and thick and extends towards the anterior roots ; (*b*) *the posterior horn* is longer and more slender. In the concavity of each crescent the grey matter sends out processes which enclose portions of the white substance.

Surrounding the grey matter on all sides is the granular white matter. It is traversed by delicate bands of medullated fibres, which extend from the extremities of the grey matter towards the outside of the cord. These fibres are the anterior and posterior roots respectively of the spinal nerves. A delicate coat of fibrous tissue—the pia-mater—encircles the cord and sends into it numerous processes. The white substance is cleft vertically by a space—*the anterior median fissure*—which extends *nearly* down to the transverse portion of the grey matter. It divides that portion of the cord which is nearest to the bodies of the vertebræ. The white matter on the posterior aspect of the cord is divided by a somewhat similar space—*the posterior median fissure*. This fissure differs from the anterior in the fact that it is narrower, that it is deeper as it extends down to the grey commissure, and that it is usually occupied by the delicate connective-tissue forming the basis of the cord called *the neuroglia*. Laterally the surrounding pia-mater penetrates the white matter at various points ; these ingrowths are somewhat more marked in the neighbourhood of the anterior and posterior roots. This portion of the cord is therefore divided by antero-lateral and postero-lateral fissures into antero-median, antero-lateral, lateral, postero-lateral, and postero-median *columns*. At the bottom of the anterior fissure is a transverse band of white substance called the anterior white commissure ; it separates the fissure from the anterior grey commissure, which in turn is separated from the posterior grey commissure by the central canal. The posterior grey commissure lies immediately above the posterior median fissure.

Under a somewhat higher power, such as a half-inch objective, it will be seen that the grey matter is composed of a finely granular matrix enclosing numerous polygonal bodies which,

in a well-prepared specimen, have many delicate branches. These bodies are the ganglion cells. They are largest and most numerous in the anterior and posterior extremities of the horns as well as round the periphery. They are least numerous in the centre and in the commissures. The granular appearance of the white matter is due to the section of a large number of medullated nerve-fibres which vary considerably in size and are embedded in a matrix of neuroglia. In transverse section these fibres consist of circular masses with a more deeply stained centre, representing the axis cylinder. In the anterior region the white matter is traversed by several bundles of non-medullated fibres, which form the anterior motor roots, whilst in the posterior region, the posterior or sensory root passes through it as a single band.

Under a still higher power, such as a $\frac{1}{6}$ inch objective, the various constituents of the cord may be considered in greater detail. (1) The white matter consists of longitudinal fibres which in transverse section measure $\frac{1}{1500}$ to $\frac{1}{15000}$ of an inch in diameter. They are finer in the posterior and postero-lateral columns than elsewhere. Each fibre can now be seen to consist of an external coating derived from the neuroglia which stains, and which surrounds an unstained portion, which again encloses a small central and deeply staining portion. The unstained part is often arranged in concentric rings. Connective-tissue corpuscles belonging to the neuroglia are sometimes found between the nerve-fibres. (2) The grey matter is made up of small non-medullated fibres, which chiefly form a dense network, continuous with the roots of the nerves ; part of these fibres are derived from the branches of the nerve-cells embedded in the network. The cells are of two kinds : (i.) *Large, branched, and nucleated*, which are most numerous in the anterior cornua, especially at their upper and outer parts. They are also found at the inner part of the base (cervix cornu) of the posterior horn, forming the *posterior vesicular column*, which is best marked in the lumbar enlargement of the cord. Lastly in the concavity of the crescent is a group of cells, occupying a projection of grey matter there and called the *tractus intermedio-lateralis ;* it is best marked in the dorsal region. (ii.) Smaller cells scattered throughout the grey

matter, but chiefly at the tip (caput cornu) of the posterior horn, in a finely granular basis, and among the posterior root fibres (*substantia gelatinosa cinerea* of Rolando). (iii.) *The neuroglia* forms the delicate connective-tissue supporting the grey and white matter of nerve structures. Its general characters are excellently seen in some parts of the pineal body. It consists of a transparent semi-fluid matrix, interspersed with delicate fibrils resembling those found in elastic tissue. The fibrils form a network, in the meshes of which lie small branched neuroglia cells. A few larger branched cells of Deiters are also present. The neuroglia forms a perivascular lymph-space for the blood-vessels, which are numerous but small.

The Peculiarities of Different Regions of the Spinal Cord.—The outline of the grey matter and the relative proportion of the white matter varies in different regions of the spinal cord, and it is therefore possible to tell approximately from what region any given transverse section of the spinal cord has been taken.

In the cervical enlargement the grey matter occupies a large proportion of the section, the grey commissure is short and thick, the anterior horn is blunt, whilst the posterior is somewhat tapering. The anterior and posterior roots run some distance through the white matter before they reach the periphery.

In the dorsal region the grey matter only bears a small relation to the white, and the posterior roots in particular run a long course through the white matter before they leave the cord; the grey commissure is thinner and narrower than in the cervical region.

In the lumbar enlargement the grey matter again bears a very large proportion to the whole size of the transverse section, but its posterior cornua are shorter and blunter than they are in the cervical region. The grey commissure is short and extremely narrow.

At the upper part of the conus medullaris, which is the portion of the cord immediately below the lumbar enlargement, the grey substance occupies nearly the whole of the transverse section, as it is only invested by a thin layer of white substance. This thin layer is wanting in the neighbourhood of the posterior nerve-roots. The grey commissure is extremely thick.

At the level of the fifth sacral vertebra the grey matter is again in excess, and the central canal is enlarged, appearing T-shaped in section, whilst in the *upper portion of the filum terminale* the grey matter is uniform in shape without any central canal.

MEDULLA OBLONGATA OR BULB.

Preparation, as for the spinal cord.

Structure.—Under a low power a transverse section through the bulb (i.) a little *below the apex of the calamus scriptorius* shows the great depth of the anterior median fissure, with the comparative shallowness of the posterior fissure. The latter fissure is bounded on either side by *the fasciculus gracilis*, which is the continuation upwards of the posterior median columns of the spinal cord. The upward prolongation of the postero-lateral column which has here expanded into the wedge-shaped *cuneate fasciculus* is situated more anteriorly than the fasciculus gracilis, and still more in front is a new band of fibres known as the *tubercle of Rolando.* The *olivary body*, which is dentate in section, is yet more anterior, and is separated from the *pyramid* by a bundle of fibres, which represents *the root of the hypoglossal nerve.*

The higher power will show that the anterior and lateral regions of the grey matter are here arranged in a *reticular formation* caused by the intersection of nerve-fibres running in longitudinal and transverse directions. Numerous multipolar ganglion-cells are scattered throughout the reticular formation.

(ii.) A section through the bulb *at the level of the fourth ventricle* shows that the anterior median fissure still remains as a deep cleft, whilst the posterior median fissure has expanded into the space which represents the fourth ventricle. A layer of grey matter, containing numerous ganglion-cells, forms the floor of the ventricle. The group of ganglion-cells nearest to the middle line upon either side is the nucleus of the *fasciculus teres.* A little external and anterior to it, *i.e.*, deeper in the grey matter, is a collection of large ganglion-cells, forming *the nucleus of the hypoglossal nerve*, from which the nerve-roots may be seen passing through the reticular formation to the point where they leave the bulb, between *the pyramid* and *the olivary*

nucleus. Still more externally are other groups of ganglion-cells, from which *the vagus* arises. This group of cells is partially subdivided by a bundle of fibres known as *the fasciculus solitarius*, which appears circular in transverse section; of the two groups, the more central and deeper is the larger. The root of the nerve passes outwards through *the reticular formation*. *The nucleus gracilis* and the *nucleus cuneatus* lie still more externally; they are portions of grey matter which extend respectively into the fasciculus gracilis and fasciculus cuneatus. *The ascending root of the fifth nerve* is situated laterally, as a well-defined bundle of white fibres.

CEREBELLUM.

The cerebellum is hardened in the usual bichromate mixture.

To stain specimens of it, the following method is recommended. Thin sections should be left in a ·01 per cent. solution of eosin for twelve hours, and then, after washing in distilled and slightly acidulated water, should be placed in a weak solution of aniline green for fifteen or twenty minutes, being afterwards passed rapidly through the ordinary reagents, and mounted as for cerebrum.

Structure.—It is divisible into (A) Cortical grey; and (B) Internal white substance.

(A) **The Cortex** is composed of (1) *The molecular layer*, the most external, consisting of a nerve network containing small multipolar ganglion-cells. The fibres of the network in the more superficial portions are nearly vertical to the surface; they are derived partly from the neuroglia, partly from the processes of the cells of Purkinje. (2) A single layer of large, spindle-shaped ganglion-cells $\frac{1}{800}$ to $\frac{1}{1000}$ inch in diameter, *Purkinje's cells*. Each cell possesses one branched process which extends into the molecular layer, where it branches dichotomously, some of the finest ramifications looping backwards to terminate in the granular layer, and an unbranched axis-cylinder process passing downwards. The cells lie in a pericellular space, and each consists of a minute network of fibrils extending into the branched processes. The nucleus is spherical and oval. (3) *The granular layer*, containing a network of minute fibrils

and dense groups of granule-like corpuscles. These corpuscles average $\frac{1}{4000}$ to $\frac{1}{2500}$ inch in diameter.

(B) *The internal white substance*, or **Medullary Centre**, consists of nerve-fibres arranged in parallel or interlacing bundles.

The neuroglia of the white matter contains rows of small cells, each with a spherical nucleus, between bundles of nerve-fibres.

The blood-vessels of the grey matter pass from the pia mater in a vertical or oblique direction, and anastomose into a uniform network. The blood-vessels of the white matter form a network with longitudinal meshes. The vessels lie in lymph channels, *the perivascular lymphatics* of His.

CEREBRUM.

Preparation.—Having carefully removed the pia mater, place small pieces from different parts of the human cerebrum in a 2 per cent. solution of ammonium bichromate for two days, after which transfer to weak, and finally to strong spirit. Take care to get vertical sections. Stain in aniline blue-black.

Structure.—Get a general view of the structure with a $\frac{1}{2}$ or $\frac{2}{3}$ inch lens, and then use a $\frac{1}{5}$ objective. With the former it will be seen that the cerebral convolutions are divisible into (A) Cortical grey portion, and (B) Medullary white substance.

With the high power the (A) **Cortical Grey portion** is seen to be composed of :

(1) *An external layer* containing a few small cells with fine processes embedded in a considerable quantity of neuroglia. This layer composes about $\frac{1}{10}$ of the whole thickness of the grey substance.

(2) *A second layer* of small, densely aggregated, pyramidal cells, provided with branching processes. This layer is of nearly the same extent as the previous one.

(3) *A third layer* of greater width, somewhat paler than the first and second layers ; it is composed of large and small pyramidal cells, arranged with their apices turned towards the surface of the convolution. The larger cells average $\frac{1}{1800}$ inch across their base. The cells are arranged in groups, and are

separated from each other by bundles of radiating nerve-fibres, each bundle being about $\frac{1}{1500}$ inch in diameter. The pyramidal cells send downwards three processes, of which the middle one forms an axis cylinder. Both cells and processes are striated longitudinally, and generally contain a little yellowish pigment.

(4) *A fourth layer* is somewhat narrower than the preceding ; it consists of small, irregularly placed, granule-like corpuscles, with delicate processes. The cells are less distinctly separated into groups.

(5) *The lowest layer* is of considerable width ; it contains, in addition to cells resembling those of the fourth layer, fusiform cells arranged vertically at the summit of a gyrus, but parallel to the surface of a sulcus. This layer gradually blends with

(B) **The White Substance**, which is composed essentially of white nerve-fibres, smaller than those of the spinal cord, and with an average diameter of $\frac{1}{10000}$ inch. In the neighbourhood of the cortex, a few non-medullated fibres can be seen.

(C) *The neuroglia*, formed of a homogeneous matrix, in which lie numerous elastic fibrils, connected into a network. With this network the branched nucleated cells of Deiters are connected.

SYMPATHETIC SYSTEM.

Harden (1) portions of the Gasserian ganglion from the sheep in chromic acid and spirit. (2) Pieces of the sympathetic nerve from the neck of the ox, in the manner recommended for medullated nerve-fibres. (3) Paccinian corpuscles by snipping out pieces of the mesentery or meso-rectum of the cat, in which they may be seen as small bodies embedded in the fat : isolate them with needles, treat with osmic acid, stain in picrocarmin, and mount in glycerine. (4) Meissner's and Auerbach's plexuses, situated the one in the submucous coat, and the other between the muscular coats of the intestine, are best prepared from the rabbit or guinea-pig. A piece of intestine, three inches in length, is distended with the juice of a fresh lemon, the ends being ligatured ; it is allowed to remain in the lemon juice for about five minutes. The ligatures are then removed, and it is washed thoroughly in water, and filled with a 2 per cent. solution of gold

chloride ; it is again ligatured, and is then suspended for half an hour in a 1 per cent. solution of gold chloride, washed thoroughly, and transferred to a 24 per cent. solution of formic acid to reduce the gold, the preparation being kept in the dark. After reduction of the gold, the intestine is of a rich reddish-brown colour : it should be again washed. Peel off strips of the outer muscular coat with forceps, and mount in glycerine. Auerbach's plexus will be seen. The plexus of Meissner is demonstrated by treating the intestine as before, and afterwards inverting it and removing the mucous coat, when portions of the submucous coat may be picked off from the muscular coats, and mounted in glycerine.

General Characters.—*The ganglion-cells* are of various shapes and sizes, generally smaller than the cells of the cerebro-spinal ganglia ; they possess a capsule, and consist of a network of fibres. There is usually one large, excentric, oval nucleus, which may, however, be double. The cells possess one or more processes, which are continuations from the cell substance, and are invested by a prolongation from the hyaline sheath. According to the number of processes, the cells are unipolar, bipolar, or multipolar. In the frog the bipolar cells are peculiar, since one of the processes appears as a 'spiral fibre' twisted round the other process, or 'straight fibre.'

The nerve-trunks contain medullated fibres similar to those already described, and non-medullated, or fibres of Remak. Remak's fibres are pale, finely fibrillated axis cylinders, invested with a hyaline sheath of Schwann provided with nerve-corpuscles.

The Paccinian bodies are oblong corpuscles connected with a medullated nerve-fibre which represents its stalk. The corpuscle consists of a number of concentric capsules. Each capsule is composed of a hyaline basement membrane, which is probably elastic, and in which are embedded fine connective-tissue fibres. On the inner surface of the basement membrane is a layer of flattened nucleated endothelial cells, which are visible after treatment with nitrate of silver. In the centre of the corpuscle is a clear mass in which lies the axis cylinder of the perforating nerve-fibre. The axis cylinder generally breaks up into two or

three branches, or it may terminate in a bud or in a pointed or fringed extremity. The corpuscles contain, between the capsules, capillary blood-vessels and a few plasma-cells.

The nerve plexuses consist of a meshwork of flat nerves, each of which is ensheathed in a delicate endothelial membrane; at the nodal points are groups of ganglion-cells, which vary in size and shape; the larger ones possess a capsule and processes. The plexus of Meissner consists of a larger and less regular meshwork than that of Auerbach. The two plexuses are connected by branches which pass through the circular layer of muscle. Each plexus gives off branches which supply the surrounding tissues.

CHAPTER VII.

BLOOD-VESSELS.

Of three kinds. (A) Arteries, (B) Veins, and (C) Capillaries.

(A) **Arteries.** — *Longitudinal* and *transverse* sections of a medium-sized artery (or vein), which has been hardened in a 1 per cent. solution of potassium bichromate, should be stained in logwood, prepared, and mounted in Canada balsam.

Good transverse sections of small arteries and veins may be seen in sections of various organs, *e.g.*, the tongue, liver or heart.

Structure.—Arteries (except those of minute size) have three coats :

1. Internal coat, consisting of (*a*) *An endothelial layer*, forming the lining of the vessel, of thin elliptical or irregularly polygonal cells, often lanceolate, with nuclei and nucleoli ; (*b*) *A sub-endothelial layer* of delicate connective-tissue, with branched corpuscles ; (*c*) *Elastic layers* of longitudinal elastic networks and 'fenestrated' membrane.

2. Middle coat chiefly consists of circular *unstriped muscle fibres*, mixed with *elastic fibres*, and a sparse amount of connective-tissue.

3. External coat (*tunica adventitia*) chiefly consists of fine and closely felted bundles of *connective-tissue*, together with longitudinal elastic tissue between them.

In the largest arteries, the middle coat consists of alternate layers of elastic tissue and unstriped muscle. In the smallest arteries (arterioles) the coats are reduced to a muscular, principally of circular fibres, and a lining endothelium.

(B) **Veins.—Structure.**—As of the arteries, with these dif-

ferences : The elastic tissue of the internal coat seldom occurs in the form of fenestrated membranes. The middle coat is thinner, contains less muscular tissue and more white connective-tissue. The external coat of some veins has a considerable amount of unstriped muscular fibre. The valves, when they are present, consist of folds of the internal coat covered on each surface with endothelium. Each fold contains a few muscular fibres and a little connective-tissue derived from the middle coat.

(c) **Capillaries.**—These are to be obtained from the pia-mater. The brain of some animal, such as a cat or dog, should be left for two days in a 2 per cent. solution of potassium bichromate, then the pia-mater may be stripped off in pieces, stained, and mounted in the usual manner. Capillaries are well seen also in the mesentery of a cat or other animal.

Structure.—The walls of the capillaries proper are formed entirely of a simple epithelial layer of flattened lanceolate cells, joined edge to edge, and continuous with the layer which lines the arteries and veins. The larger capillaries have an outside structureless or finely fibrillated coat. In rather larger vessels (small arteries and veins) there is added, outside the epithelium, a thin layer of unstriped muscular fibre.

CHAPTER VIII.

ALIMENTARY CANAL AND ITS APPENDAGES.

TONGUE.

Vertical sections of a rabbit's or cat's tongue, which has been hardened in equal parts of $\frac{1}{2}$ per cent. chromic acid and spirit, should be stained and treated in the usual manner.

Sections of the *papilla foliata* which is found on either side of the base of the rabbit's tongue should also be treated in a similar manner. The tongue furnishes excellent material for double or triple staining.

Structure.—The tongue, for histological purposes, may be considered to consist of a mucous membrane covering voluntary muscles and glands.

The mucous membrane of the dorsal surface is elevated into numerous papillæ, which are of varying shape. Each papilla is covered by stratified epithelium, the superficial layers of which in the carnivora are converted into dense plates of chitinous material. The papillæ, according to their shape, are known as the filiform, the fungiform, and the circumvallate.

(*a*) *The filiform papillæ* are the most numerous, and are found upon the anterior and lateral surfaces of the organ. They are minute elevations of the corium covered by epithelium, their apices being frequently subdivided into secondary papillæ.

(*b*) *The fungiform papillæ* resemble in shape unexpanded mushrooms or 'puff-balls.' They consist, like the filiform papillæ, of areolar tissue enclosing a loop of capillaries, and they are covered with a layer of squamous epithelium. These papillæ are most numerous at the apex and near the margins of the tongue.

(*c*) *The circumvallate papillæ* are situated at the posterior portion of the dorsal aspect of the tongue, and are much larger than either the filiform or fungiform papillæ. In section they appear as flat-topped elevations of the mucous membrane, with a deep depression upon either side. The duct of a serous gland frequently opens into the bottom of the depression. They are covered with flattened epithelium like the other varieties of papillæ, but at their sides some of the epithelial cells have become modified into *taste-buds*.

(*d*) *The papilla foliata* is an elongated oval patch seen upon either side of the rabbit's tongue. It consists of a number of parallel invaginations of the mucous membrane. The epithelium thus invaginated contains numerous ovoidal or flask-shaped bodies identical with those found in the human circumvallate papillæ : they are *the taste-buds*. Each of these bodies consists of a layer of flattened epithelial cells, arranged round, and forming a covering for, a bundle of central cells. The central cells are spindle-shaped, one extremity projecting through the aperture left by the external cells, whilst the other extremity is branched and extends into the submucous layer, where it probably becomes connected with a terminal fibre of the glosso-pharyngeal nerve.

Numerous *mucous secreting glands* are found in the tongue. Of these the largest are the glands of Nuhn, situated at the tip. *Serous glands* are also present, they open into the trenches of the circumvallate papillæ. *Lymphoid tissue* is found in large quantity in the submucous tissue at the posterior part of the tongue, in some cases forming follicles ; numerous crypts and recesses, too, are found, the walls being studded with nodules of lymphoid tissue.

The mucous membrane on the under surface of the tongue consists of a thin layer of stratified pavement epithelium, with a somewhat dense submucous tissue. On the dorsum of the tongue the submucous coat is incomplete and scanty, though it contains a large number of blood-vessels.

The muscular portion of the tongue consists of longitudinal, transverse, and vertical fibres of striated muscles ; considerable quantities of fat and gland tissue are found between the bundles of muscle.

THE TEETH.

These structures may be examined (*a*) By means of sections of the hard tooth : Grind down the tooth on both sides till it is quite thin, then mount in hard Canada balsam, so as to retain the air in the various cavities.

(*b*) By means of sections of the softened tooth: Place the tooth in 10 per cent. solution of hydrochloric acid till it is quite soft, then immerse in spirit ; by this means the structure of the dentinal substances may be investigated ; or place the tooth (preferably broken across) in a saturated solution of picric acid until it is quite soft. Complete the hardening in spirit, changing the spirit so long as it becomes tinged with the picric acid. This method of preparation will preserve the pulp and odontoblasts.

To demonstrate the pulp, break a freshly extracted tooth, and immerse it in osmic acid $\frac{1}{5}$ per cent. for twenty-four hours.

To show the development of teeth, place the lower jaw of a fœtal rat, dog, or kitten, from which the muscles have been removed, in $\frac{1}{6}$ per cent. chromic acid for seven days, then remove to weak spirit for twenty-four hours, and finally to strong spirit till required.

(*c*) The dentinal sheath lining the tubules may be isolated by boiling for ten minutes in strong sulphuric acid. In each case stain in hæmatoxylin.

[*Note.*—It is best to buy prepared sections of teeth.]

Structure.—(1) A tooth consists most externally, and above the gum, of *enamel*. In the recently cut tooth, which has not been much used, there exists above the enamel a covering of epithelial or horny nature (Nasmyth's membrane), which is structureless, and has a thickness of $\frac{1}{3000}$ to $\frac{1}{1000}$ inch. The enamel covers the crown and neck of the tooth ; it is an epithelial product, consisting of closely aggregated polyhedral cylinders forming the enamel fibres (prisms or columns). The set fibres are crossed by a number of darker lines, arranged in concentric layers, 'contour lines.' In transverse section, the enamel fibres are seen to be six-sided prisms, with an average diameter of $\frac{1}{5000}$ inch.

(2) *The Dentine*, or ivory, covers the body and root of the

tooth ; on the surface it lies immediately below the enamel. The dentine consists of a compact bone-like substance, which contains no bone corpuscles, and is permeated by dichotomously dividing canals, the dentinal-tubules, which average $\frac{1}{4500}$ inch in diameter. The dentinal-tubules run perpendicularly to the surface of the pulp cavity, into which they open by their lower extremities. The tubules present a proper wall, consisting of a membranous tube, and each contains a process of protoplasm from the superficial layer of the pulp-cells. Examined under a lower power, the tubules are seen to form two or three gentle curvatures, which give rise, when a number of tubules are seen together, to a series of concentric lines (lines of Schreger). Certain interglobular spaces, due to imperfect deposition of salts, are also frequently seen in the dentine.

(3) *The crusta petrosa*, or cement, invests the portions of the tooth which are not protected by enamel. It closely resembles bone in its histological appearances, except that the lacunæ and canaliculi are larger and more irregular. When the cement is very thick, it may contain vascular channels, which are comparable with Haversian canals. The perforating fibres of Sharpey are present in considerable numbers in the cement.

(4) *The pulp* occupies the central cavity of the tooth ; it consists of jelly-like connective-tissue, in which run nerves and blood-vessels. The outermost layer of cells, forming the pulp, are elongated in form, the bodies somewhat resembling columnar epithelium cells. This layer forms the membrana eboris of which each cell is an odontoblast. The odontoblasts send off one or more processes, which run in the dentinal-tubules ; processes which connect the cells together laterally, and processes which unite the cells to others, lying more deeply.

(5) *Osteodentine*, or secondary dentine, is the hard substance deposited on the inner surface of the dentine, which is produced by the gradual conversion of the pulp.

Development.—(1) The first rudiment of a tooth appears as a solid prolongation of the stratified epithelium, which grows downwards from the surface into the mucous membrane. This process of epithelium is the *primary enamel organ.*

(2) The enamel organ becomes invaginated at its deep end by

a mass of tissue derived from the mucous membrane, called the embryonal tooth papilla. The primary enamel organ is thus converted into the *enamel cap* covering the tooth papilla.

(3) *The papilla* is vascular, and is composed of a network of nucleated cells; it forms the pulp, and by means of its odontoblasts forms the dentine.

(4) *The odontoblasts* appear on the papilla as a peripheral stratum of large cells arranged vertically.

(5) *The dentine* is formed by the calcification of the substance which surrounds, and is perhaps derived from, the distal extremities of the odontoblasts, whilst

(6) *The dentinal fibres* are processes of the odontoblasts.

(7) *The tooth sac*, or the mucous membrane which immediately surrounds the enamel cap and tooth papilla, gradually grows over the former, and separates it from its connection with the surface epithelium.

(8) The *enamel cap* consists externally of (*a*) columnar cells, more internally of (*b*) polyhedral cells, followed by (*c*) flattened epithelial cells in the centre, and again of (*d*) polyhedral, with (*e*) columnar cells most internally—*i.e*, nearest to the tooth papilla.

(9) The enamel cap is limited both externally and internally by *a membrana propria.*

(10) The enamel cap becomes divided into an inner and outer membrane by the transformation of the middle layer (8*c*) into a transparent tissue. *The inner membrane* is composed of columnar cells, the enamel cells, in contact with the dentine; each is a long hexagonal prism, and is nucleated at its lower part. Outside the layers of enamel cells are one or more rows of small polyhedral cells, forming the stratum intermedium. *The outer membrane* is composed of several layers of epithelial cells.

(11) *The enamel* is formed by the enamel cells of the inner membrane elongating at their distal extremities; the elongated portion is transformed directly into enamel.

(12) The cells of the stratum intermedium are used for the regeneration of the enamel.

(13) The cells of the outer epithelium produce the *enamel cuticle.*

(14) *The cement* is formed from the tissue of the tooth sac in exactly the same way as sub-periosteal bone is developed.

(15) During the stage of the primary enamel organ (*a*) a lateral process grows out from the epithelial cells, which represents the rudiment of the enamel organ of the permanent tooth (*sac of reserve*).

(16) The *permanent* teeth are developed on exactly the same plan as the deciduous set.

SALIVARY GLANDS.

Sections of the submaxillary gland of a cat or dog, and of the parotid of a rabbit or dog, should be made in various directions after hardening in spirit.

Structure.—The salivary glands are compound tubular glands enveloped in an incomplete capsule. The capsule consists of fibrous tissue which sends septa into the substance of the gland. The septa support the blood-vessels, lymphatics, nerves, and ganglia, and they divide the glandular substance into lobes, lobules, alveoli, and acini. Each lobule is made up of the convolutions of the main division of a duct bound together with connective-tissue. The convoluted parts are lined by and almost filled with a single layer of columnar cells (*salivary cells*) enclosing a nucleus. These cells, when isolated, are not unfrequently found to be branched. The basement membrane of the tubes consists of branched and flattened cells, and between it and the salivary cells are found, here and there, but not in the parotid, granular semilunar bodies, *the semilunes of Heidenhain.* The smallest divisions of the ducts have a relatively small lumen, and are lined near the convolutions with flattened epithelium, and then with nucleated columnar cells which present a longitudinal striation. The larger ducts acquire an external coating of connective-tissue, and are lined with a single layer of columnar epithelium. In the walls of the largest ducts are unstriped muscular fibres.

The salivary glands are divided into (*a*) *Mucous glands*, in which the alveoli are large, and the cells are (1) mucous cells, transparent and columnar, with their pointed extremities applied to the membrana propria ; the cells are imbricated ; the nucleus

is much compressed, and is near the membrana propria. (2) The semilunes of Heidenhain, or the crescents of Giannuzzi, semilunar groups of cells situated here and there between the mucous cells and the membrana propria. The cells are small and polyhedral, with a spherical nucleus. The submaxillary and orbital glands of the dog and the sublingual gland of man are of the mucous type.

(*b*) *Serous glands*, in which the lumen of each alveolus is small, and the epithelium consists of a single layer of short columnar cells, each with a spherical nucleus situated at the periphery of the cell. The parotid gland and the greater part of the submaxillary gland of man and the guinea-pig, as well as the submaxillary and orbital glands of the rabbit, are serous glands.

(*c*) *Muco-salivary glands*, such as the submaxillary gland of man and the guinea-pig, are formed by the mixture of the mucous and serous types of glands.

TONSILS.

Sections made from a tonsil which has remained for a week in $\frac{1}{6}$ per cent. solution of chromic acid, and subsequently in spirit, should be stained with hæmatoxylin and mounted in Canada balsam. An enlarged tonsil which has been removed from a child will answer the purpose.

Structure.—A tonsil consists of an elevation of the mucous membrane presenting upon its surface fifteen orifices leading into crypts or recesses, in the walls of which are placed nodules of lymphoid tissue. These nodules are enveloped in a less dense lymphoid or adenoid tissue which reaches to the mucous surface. The mucous surface is usually covered with squamous epithelium, and may present rudimentary papillæ which are then formed of adenoid tissue. The tonsil is bounded by a fibrous capsule. Into the crypts open a number of ducts of mucous glands.

ŒSOPHAGUS.

Small pieces of the œsophagus (both of the upper and lower parts) of a dog should be hardened in chromic acid and spirit.

Structure.—Of three coats : (1) An *external* or *muscular coat* consists of two layers, longitudinal and circular, the former or external layer at the commencement being disposed in three fasciculi, one in front and one on each side. At the upper end of the œsophagus, the muscular coat is red, and consists of striated muscle ; lower down it becomes paler, and the fibres are mostly unstriated.

(2) A *submucous coat* consists of areolar tissue, and contains mucous glands (which are best seen in sections from the lower part) and whose ducts pass inwards to open on the mucous membrane.

(3) A *mucous coat* which is firm and wrinkled, provided with minute papillæ, and covered with a thick layer of stratified epithelium. It is separated from the submucous coat by a layer of unstriated muscular fibres, longitudinally arranged, which is partially imperfect as a layer above, but complete below (*muscularis mucosæ*).

The *arteries* situated in the submucous tissue give off branches which form a network of capillaries in the upper part of the mucous coat ; from this network loops are given off to supply the papillæ.

STOMACH.

The stomach of a cat or dog may be used. After removal from the recently killed animal, the organ should be turned inside out, and washed with a gentle stream of weak bichromate of potash or spirit. Pieces of the mucous membrane from different parts should be snipped off with a sharp pair of scissors, and placed in strong alcohol. Pieces of the whole thickness of the viscus should be cut off ; these may be hardened in weak chromic acid, or in chromic acid and spirit mixture.

Sections must be cut both vertical and parallel to the surface at different depths.

To demonstrate the structure of the glands of the mucous membrane, some sections from each part should be stained in logwood, and others in aniline blue ·5 per cent. solution, as the aniline will stain the peptic cells very deeply, and so differentiate them from the cubical central cells. This is especially

evident in horizontal sections of the peptic glands. The aniline tinted specimens must be passed through slightly acidulated water, as usual in aniline staining, before they are placed in spirit.

Structure.—The stomach is made up of four coats :

(1) The *mucous* or *internal coat* is smooth, soft, pulpy, and pink in colour, becoming grey soon after death. Thickest at pylorus, thinnest at the great curve. Loosely connected with the muscular coat by means of the submucous tissue, and so presenting temporary ridges (rugæ) when the organ is contracted. It consists almost entirely of small tubular glands, arranged close to and parallel with each other, varying in diameter from $\frac{1}{500}$ to $\frac{1}{360}$ of an inch, and in length from $\frac{1}{60}$ to $\frac{1}{20}$ of an inch, lined to a variable extent by columnar epithelium, which also covers the whole of the mucous membrane.

The tubular glands are for the most part simple, except near the pylorus, where they become larger, longer, and branched. The glands consist of a basement membrane formed of branched stellate cells joined edge to edge, and sending processes on the one hand to join the retiform tissue of the mucous membrane, and on the other to support the gland-cells. The glands are of two kinds, differing chiefly in the character of the cells and of their secretion. The one, the so-called '*mucous* or *pyloric glands*,' are often branched, are confined to the pylorus, and are lined throughout by columnar epithelium; but towards the 'fundus,' or closed extremity of the gland, the cells tend to become cubical. The other, the *peptic glands*, are distributed throughout the whole of the mucous membrane, except at the pylorus, but are most typical perhaps towards the cardiac end; they are less often branched, but two glands generally open into one duct, which occupies a third of the whole length of the gland. The lower end, or fundus, is somewhat dilated, and sometimes slightly curved. The duct is lined with columnar epithelium; its middle third contains two distinct kinds of cells; outside, large granular cells with small nuclei, bulging out the basement membrane, and making irregular the outline of the tubes, called the *peptic* or *parietal cells*;* and inside, a layer of smaller finely

* It may be as well to remind the student that the so-called 'peptic cells' are no longer thought to secrete the pepsin.

granular cubical cells, *central cells*, which bound a small lumen. In the lower third, or fundus, the parietal cells do not form a continuous layer, but occur here and there irregularly ; the remainder of the tube is filled with cubical central cells, which leave a very small lumen unoccupied. The cubical cells closely resemble those lining the fundus of the pyloric glands.

Between and beneath the glands is a quantity of delicate connective-tissue, forming the mucous membrane proper, which here and there is collected into small masses somewhat resembling the solitary follicles of the intestine. A double layer (circular and longitudinal) of unstriated muscles (*muscularis mucosæ*) separates the mucous membrane from the submucous coat.

(2) The *submucous coat* consists of areolar tissue with some fat, together with blood-vessels and lymphatics ; small nerve ganglia and fibres are also found in it.

(3) The *muscular coat* consists of three layers of unstriated fibres, externally of longitudinal, then of circular, and internally of oblique fibres ; the circular layer is the only complete one. Between the layers may be found *plexuses of nerves*.

(4) The *serous coat* is the peritoneal covering of the organ.

The *arteries*, after penetrating the muscularis mucosæ, break up into capillaries in the mucous membrane, which form a more or less elongated meshwork around the glands. Near the surface the meshwork is very dense, and forms a well-marked superficial layer beneath the epithelium.

SMALL INTESTINE.

To study the epithelium in the fresh state, a scraping from the mucous membrane of the intestine of a recently killed animal may be teased and mounted in saline solution.

For the purpose of studying the relations of the various structures in the mucous membrane—*e.g.*, villi, Brunner's and Lieberkühn's glands, Peyer's patches, etc.—small pieces from each part of the intestine of the cat, dog, or rabbit should be hardened in weak chromic acid or chromic acid and spirit mixture, and cut into sections in various directions.

To demonstrate the large lymphatic sinus surrounding the

follicles comprising a Peyer's patch, the ileum is used, and the sinus is injected with Berlin blue by the puncture method, whilst ·5 per cent. solution of silver nitrate will demonstrate the endothelial lining of the vessel.

To demonstrate the absorption of fat by the villi, a part of the intestine of an animal recently fed on fatty food should be ligatured and placed in Müller's fluid ; in about a week's time pieces may be placed in osmic acid for twenty-four hours, and should afterwards be replaced in the solution.

For preparation of Meissner's plexus and the ganglia of Auerbach, see p. 88.

Structure.—There are four coats: (1) The *mucous coat* possesses:

(*a*) *Valvulæ conniventes*, which are large, naked-eye permanent folds or crescentic projections running transversely to the axis of the intestine, and containing the submucous coat. They first appear in the duodenum, not far from the pylorus ; are largest in the duodenum and upper half of the jejunum, and then gradually become smaller, until they disappear about the middle of the ileum.

(*b*) *Villi* are small processes, closely set on every part of the small intestine, over the valvulæ conniventes, as well as between them. They are conical and flattened in form, sometimes cylindrical, or with the free end clubbed. Largest in the duodenum and jejunum, in length varying from one-fourth to one-third of a line ; smaller, shorter, and fewer in ileum. They consist of projections of the mucous membrane, being covered with columnar epithelium, enclosing blood-vessels, lymphatics, and muscularis mucosæ, bound together by fine retiform tissue, which also forms the basement membrane and encloses numerous lymphial corpuscles.

(*c*) *Crypts of Lieberkühn* are very numerous small tubular glands, existing everywhere in the small intestine ; they are lined with columnar epithelium.

(*d*) *Brunner's glands* are smaller compound tubular glands found in the first half of the duodenum ; lying in the submucous coat, their ducts pass through the mucous coat.

(*e*) *Peyer's patches or glands*, or lymphatic follicular glands

occur either solitary or collected ('agminated') into oblong patches. When solitary, they are found everywhere in the small intestine, both between and upon the valvulæ conniventes ; when agminated to form Peyer's patches they occur in the ileum, especially at its lower part, lying in the long axis of the intestine opposite the attachment of the mesentery.

(2) The *submucous coat* resembles that of the stomach, as does also

(3) The *muscular coat*, but this has no oblique fibres.

(4) The *serous coat* of the duodenum is partially incomplete.

The arteries passing through the muscularis mucosæ give off numerous capillaries which form a network around the crypts of Lieberkühn ; the artery which passes into the villus generally ascends to the apex, and then breaks up into a dense plexus of capillaries, which spreads over the apex and base. The capillaries are always situated in the periphery next the epithelium. There are generally one or two veins developed from the capillaries of the villus. *The lymphatic* of each villus consists of a single central vessel, or of two such vessels anastomosing with each other.

LARGE INTESTINE.

The large intestine is similar in structure to the small intestine, but with the following differences :

(1) *The mucous coat* has neither villi, Brunner's glands, nor valvulæ conniventes, and its crypts of Lieberkühn are longer, more numerous, and are placed more closely together. The lymphoid follicles are always solitary.

(2) *Muscular coat*—in the colon and cæcum the longitudinal layer is collected into three flat bands, which causes the intestinal wall to be puckered into 'sacculi.'

(3) *The serous coat* of the colon and upper part of rectum is developed into small projections containing fat (appendices epiploicæ). It is incomplete in some parts.

THE PANCREAS.

The pancreas of a recently-fed dog should be taken, cut into pieces about the size of a small hazel-nut, and placed at

once in absolute alcohol to harden. For the sake of comparison, another pancreas from a fasting dog should be treated in a similar manner. Sections should be stained and prepared in the usual way. Unless hardened in alcohol, the gland is very likely to become useless (from self-digestion ?) for microscopic purposes.

Structure.—The capsule and septa, as well as the blood-vessels and lymphatics, are arranged as in the salivary glands ; the gland is, however, looser and softer, and the lobes and lobules are less compactly arranged.

The larger ducts possess a very distinct lumen, and a membrana propria lined with columnar epithelium cells which are longitudinally striated, but are shorter than those found in the ducts of the salivary glands.

In the smaller ducts the epithelium is short and the lumen is smaller.

The *intermediary ducts* opening into the alveoli possess a distinct lumen, with a membrana propria lined with a single layer of flattened elongated cells.

The alveoli are branched and convoluted tubes, with a membrana propria and a single layer of columnar cells. The cells consist of an outer part nearest the membrana propria, which is homogeneous, and stains the more deeply ; and an inner, more granular, and less readily stained portion. The alveoli do not contain the semilunes of Heidenhain and have no distinct lumina, their places being occupied by fusiform or branched cells.

THE LIVER.

Small portions of the fresh liver of a pig, rabbit, or puppy should be steeped for four or five days in a 2 per cent. solution of potassium bichromate, and then for one or two days in methylated spirit. Sections should afterwards be cut and treated as usual.

It is well, also, to mount sections of a liver which has been injected through the portal vein with 2 per cent. solution of Berlin blue, and then hardened in spirit, and also, if possible, of liver which has been injected through (*a*) the bile-duct, under very low pressure, and (*b*) the hepatic artery.

Structure.—It has a serous and fibrous coat. The former is absent from the posterior border, and from the portal fissure, where the latter, which elsewhere is thin, is most developed. A strong sheath of areolar tissue (' *Glisson's capsule* ') surrounds the vessels of the organ as they ramify in it, and, at the transverse fissure, becomes continuous with its fibrous coat.

The liver substance proper consists of lobules, which are closely packed polyhedral masses more or less distinct, arranged around the sides of the branches (*sublobular*) of the hepatic veins, and connected to them by minute veins which begin in the centre of the lobules (*intralobular veins*).

Each lobule consists of a mass of compressed spheroidal or polyhedral nucleated and nucleolated cells, measuring from $\frac{1}{1000}$th to $\frac{1}{800}$th of an inch in diameter, often containing oil-globules. Surrounding the lobules is a variable amount of fine connective-tissue, in which is contained a minute branch (*interlobular*) of the portal vein, a branch of the hepatic artery and of the hepatic duct, together with minute lymphatic vessels covering them. The lobules are distinct when the interlobular tissue forms complete septa around them ; if the septa are incomplete, as is usually the case in man, the lobules become confluent. Fine fibrous tissue surrounds the interlobular vein, and a delicate supporting network of flattened, branched corpuscles exists within the lobule between the cells and the blood-capillaries. Between the columns of the cells run the radicles of the hepatic vein which open into the intralobular vein, and between the cells begin the radicles of the hepatic duct. Whether these radicles of the bile capillaries have a definite membrana propria is undetermined. The interlobular *bile-ducts* are endothelial tubes with a large lumen, lined with columnar epithelium. The larger ducts are surrounded with circular unstriped muscle-cells, and have a distinct mucous membrane of loose connective-tissue lined with columnar epithelium, and containing mucous tubular glands. The *lymphatics* of the lobule originate in the spaces around the capillaries of the lobules. The branches of the *hepatic artery* run between the lobules with the interlobular veins ; in parts they surround the veins as a plexus ; the arterial branches frequently anastomose with each other, and give off

capillaries to supply the surrounding connective-tissue and vessels, the bile-ducts receiving numerous branches. The ultimate capillaries enter the lobules, where they form a plexus. The blood from the artery is carried away by a special set of veins which open into the interlobular veins ; none of it passes into the intralobular veins.

The structure of the **Gall Bladder** is similar to that of the large hepatic ducts, but the *mucous membrane* is thicker, and is thrown into folds and villous projections. *The muscular coat* also is thicker, and is surrounded by *connective-tissue* and an outer layer of *peritoneum*.

CHAPTER IX

RESPIRATORY TRACT.

EPIGLOTTIS.

THE human epiglottis, removed as soon as possible after death, should be used. It should be cut into small pieces, and placed in the chromic acid and spirit mixture until it is sufficiently hardened. Transverse and vertical sections should be well stained in hæmatoxylin, and mounted in the ordinary way.

Structure.—The epiglottis consists of a supporting cartilage of the elastic variety, enclosed in a fibrous perichondrium, and covered on both sides with mucous membrane.

The *anterior surface, i.e.*, the one towards the tongue, is covered by mucous membrane which hardly differs from that of the pharynx. This membrane consists of fibrous tissue, elevated towards the surface, in the form of rudimentary papillæ, and covered with several layers of squamous epithelium. In it ramify the capillary blood-vessels, and in its meshes are a large number of lymphatic channels. Under the mucous membrane in the less dense fibrous tissue, or submucosa, are a number of tubular mucous glands.

The *posterior or laryngeal surface* is covered by a mucous membrane which is similar in structure to the above, but the epithelial coat is thinner, the strata of cells being less numerous. The papillæ are fewer and less distinct. The proper substance of the mucous membrane appears to be in great part adenoid tissue, which here and there is collected into distinct masses. The glands of the posterior surface are smaller but more numerous than those on the anterior surface. In many places the

glands which are situated nearest to the perichondrium are directly continuous through apertures in the cartilage with those on the other side, and not unfrequently the ducts of the glands from one side of the cartilage pass through and open upon the mucous surface of the other. Occasionally the epithelium of the posterior surface is columnar.

LARYNX.

The structure of the larynx closely resembles that of the epiglottis on the one hand, and that of the trachea on the other.

The framework is hyaline cartilage enclosed in a fibrous sheath, and covered entirely by a mucous membrane. The epithelium covering this membrane is columnar ciliated, of which some of the cells are goblet-cells, except over the upper part of the false vocal cords, the arytenoid cartilages, the true vocal cords, and immediately below, where there are stratified squamous cells. There is a distinct basement membrane under the epithelium.

The mucosa is a dense fibrous tissue containing a large quantity of adenoid tissue ; it is here and there separated from the submucosa, in which lie the glands, by a thin layer of elastic fibres.

The submucosa is scanty near the true vocal cords, and contains no glands ; elsewhere it is distinct, and in it, as is generally the case with mucous membranes, the large vessels and nerves split up for the supply of the superficial structures. Taste-goblets are found in the epithelium on the posterior surface of the epiglottis, in that covering the ary-epiglottidean folds, and the inner surface of the arytenoid cartilage, and also on the true vocal cords.

TRACHEA AND LUNG.

Distend the lungs of a recently-killed rabbit or cat, through the trachea, with $\frac{1}{6}$ per cent. solution of chromic acid, tie up the trachea, and immerse it in a large quantity of chromic acid of similar strength. Change the solution for one of a $\frac{1}{4}$ per cent. after two days ; in a week cut in pieces, and remove to methylated spirit.

Before cutting sections it is necessary that the embedding mass shall have thoroughly penetrated into and filled up the interstices of the tissues, and so it is best to place the piece of lung to be embedded in the wax mass when it is quite hot. In some cases it is as well to stain the lung, as a whole, with hæmatoxylin, and pass it through alcohol and oil of cloves before embedding. Unless the interstices are filled up, it is almost impossible to cut thin sections.

To free the cut sections from wax, pass them through oil of turpentine before putting them into oil of cloves.

A better method is to soak the lung in gum, and then to cut sections of it by means of the freezing microtome; afterwards removing the gum by placing the sections in warm water.

Thin sections of lung injected through the pulmonary artery with Berlin blue, and through the trachea with $\frac{1}{2}$ per cent. solution of silver nitrate, should be made and treated in the usual manner. Sections of trachea should also be prepared.

Structure.—(1) *Of the trachea.* (*a*) *An elastic framework* of incomplete rings or hoops of hyaline cartilages, 16 to 20 in number; each presents a curve of rather more than $\frac{2}{3}$ of a circle. These rings are held together by a strong fibrous membrane, more or less elastic, which not only occupies the interval between them, but is prolonged over their outer and inner surfaces; behind, where the cartilage is incomplete, the fibrous membrane is strengthened by a continuous layer of unstriated muscle, chiefly arranged transversely. (*b*) *A submucous coat* of areolar tissue and fat is also present; it contains immediately beneath the mucous membrane longitudinal fibres of elastic tissue, which are for the most part collected into bundles. Tubular mucous glands are found in this coat, and also upon and beneath it. (*c*) *A mucous membrane* containing a large amount of lymphoid tissue; under the epithelium is a basement membrane of flattened cells, which send up processes to the epithelium. In the deeper parts are many elastic fibres. On the surface are several layers of epithelium, of which the more superficial are columnar and ciliated, often branched below to join the connective-tissue corpuscles. Between the branched ends of these cells are smaller elongated

cells, prolonged upwards towards the surface, and downwards to the basement membrane. Beneath these are one or more layers of irregularly shaped cells.

(2) *Of the bronchi*, as of the trachea.

(3) *Of lung.* The tissue is made up of lobules attached to the minute divisions of the air-tubes, by which they are held together, as well as by blood-vessels and interlobular tissue. The lobules, although adherent, are quite distinct; the structure of each represents that of the entire lung, and consists of a minute *air-tube* with terminating *air-cells*, lined with tesselated *epithelium*, together with the pulmonary and bronchial blood-vessels, lymphatics, nerves, and areolar tissue. The principal divisions of the bronchi divide, generally dichotomously, into branches running in all directions, which never anastomose, but terminate separately in the lobules. Within the lobules each bronchial tube finally ends in small recesses (air-cells, alveoli, or vesicles), having previously lost its cylindrical form, from being beset with similar air-vesicles on all sides; in this condition the tube becomes what is called an *infundibulum*. The structure of the air-tubes gradually changes as they become smaller. The cartilages become irregularly shaped plates of different sizes, scattered over the sides of the tubes, gradually becoming fewer, and finally disappearing before the infundibulum is reached. The fibrous coat extends to the smallest tubes, by degrees becoming simply areolar. The mucous membrane becomes thinner, but retains its former epithelium, the cells becoming very short columnar in the smallest bronchi. The longitudinal elastic bundles are traceable into the smallest tubes. The muscular fibres ultimately form a continuous circular layer inside the cartilaginous plates. The walls of the infundibula consist of (*a*) unstriped muscle arranged circularly; (*b*) a network of elastic fibres; (*c*) fibrous tissue and connective-tissue cells; (*d*) a dense meshwork of capillary blood-vessels; (*e*) small polyhedral cells and large flattened cell-plates. These plates vary in shape and size according to the amount of distension of the air-vesicles; they are best seen in lungs stained with nitrate of silver. (*f*) Between the cell-plates *pseudo-stomata* may be found; *i.e.*, larger or smaller circular or angular openings similar to the stomata found in serous mem-

branes; they lead into the lymph-canalicular system of the alveolar wall.

The blood-vessels constitute a dense capillary plexus upon the alveolar septa : in the contracted lung the capillaries are very sinuous and close together, whilst in the distended lung they are straighter and further apart. Near the pleura and bronchi the capillaries anastomose with the capillaries of the bronchial artery. The larger arterial and venous branches are situated in the interlobular connective-tissue, which is continuous with their outer coat. *The lymphatics* are arranged in three systems. (*a*) The subpleural lymphatics, forming a dense plexus whose meshes mostly correspond with the outlines of the alveoli. (*b*) The perivascular lymphatics, whose vessels accompany the branches of the pulmonary artery and vein. (*c*) The peribronchial lymphatics, remaining in the outer coat of the bronchi, and anastomosing freely with the perivascular lymphatics.

CHAPTER X.

SKIN AND APPENDAGES.

SMALL pieces of skin from various parts—*e.g.*, from palm of hand, fingers, or toes, scalp, scrotum, and general surface, should be hardened in equal parts of chromic acid, $\frac{1}{2}$ per cent., and of methylated spirit, for a week, changing the liquid on the second, fourth, and seventh days, and then removing to spirit until required. Sections may be made in various directions (cutting towards the epidermis is the easiest way), stained, prepared, and mounted in the usual manner.

Double staining, with picrocarmin as well as with logwood, is recommended.

Injected specimens of skin may be prepared by injecting 2 per cent. Berlin blue solution into the main artery of a limb of a dog, or one of the upper extremities of a fœtus.

Structure.—The skin consists of two parts :

1. *Epidermis, or external skin*, which is made up of several more or less distinct layers. (*a*) The most superficial horny layer (stratum corneum) varies in thickness, and is composed of layers of flattened epithelium, which show nuclei only after treatment with softening reagents, *e.g.*, caustic potash. (*b*) The next layer (stratum lucidum) is generally homogeneous and thin ; it is composed of closely packed scales. (*c*) A layer of granular cells (stratum granulosum), flat, spindle-shaped, and nucleated, which stain deeply in logwood. (*d*) Finally, the Malpighian layer (rete Malpighii, or rete mucosum), consisting of stratified epithelium, the deepest layers of which are columnar, the next more or less cubical 'ridged' cells, connected together

by filaments or prickles, and most superficially are layers of flattened cells.

2. *Internal, or true skin* (*corium or cutis vera*), is made up of dense areolar tissue, in which is found, lying deeply, a good deal of fat; muscular fibres occur in the neighbourhood of hairs; they exist as a distinct layer in the subcutaneous tissue of certain parts, *e.g.*, scrotum, penis, areola of the nipple, etc. In the superficial part of the corium are numerous conical elevations or papillæ, which are received into corresponding pits in the epidermis; they are most developed where sensation is most acute. The subjacent or reticular part of the corium contains hair follicles, with sebaceous glands, and sweat glands.

Nerves and blood-vessels are numerous: the former, ending in the Malpighian layer in a delicate network, and supplying certain of the papillæ, form special endings (end bulbs and tactile corpuscles); the latter form near the surface a dense network of capillaries with rounded polygonal meshes.

GLANDS.

A. **Sweat-glands** are found distributed throughout the skin generally, and are exceedingly numerous. Each gland consists of a long duct which passes through the skin in a more or less wavy manner, to open on the surface, and a coiled gland proper contained in the subcutaneous tissue. The duct of the gland consists of a narrow tube made up of a basement membrane (homogeneous), lined with several layers of small cubical epithelial cells limited internally by an endothelial membrane, which encloses a lumen, generally circular in form. The gland proper is made up of the coils of the duct, differing in number according to situation. The coils nearest the duct proper differ little in structure from the above, but the remainder of the gland (distal portion) is found to have a single layer of columnar cells lining it, instead of several layers of small cells; the internal limiting membrane is less distinct, and the membrana propria, or basement membrane, is strengthened and made thicker by an internal layer of longitudinal unstriped muscular fibres. The glands in the neighbourhood of the anus are exceedingly large.

B. **Ceruminous glands** are similar in structure to the sweat-glands elsewhere, but the gland proper is throughout like that of the distal part of the sweat-gland, as described above.

C. **Sebaceous glands**, as a rule, open into the neck of hair follicles. Each gland is composed of a short duct, which branches into several dilated alveoli, which may each be further subdivided. The duct is lined with two or three layers of small nucleated cells, and each alveolus is lined with smaller cubical nucleated cells. The remainder of the alveolus is filled up with cells increasing in size towards the centre, and filled with fat. These central cells have been produced by the division of the lining cells, and, as they reach the centre of the alveolus, pass into the duct, lose their nuclei, and, discharging their fatty contents externally, shrivel up, and are discharged in the sebaceous secretion.

HAIR.

Hairs may be seen in sections of the skin or scalp, especially well in doubled-stained sections which have been placed in picrocarmin and hæmatoxylin. Single hairs may be examined in any reagent, but best in caustic potash. Transverse sections are made in the ordinary operation of shaving, and may be examined in saline solution.

Structure.—The free extremities of hairs above the skin are pointed, the attached extremities are received into follicles in the corium ; between the extremities is the shaft. The follicular end is bulbous, and cased in a compound sheath.

A hair itself is made up of (*a*) an external covering of thin scales (cuticle) ; (*b*) a cortical substance made of coloured horny matter ; and finally (*c*) the medulla or pith, which is absent in some hairs.

The bulb of the hair rests upon and overlies an elevation of the follicle (papilla), which is composed of undeveloped nucleated connective-tissue corpuscles and a few fibres.

The sheath of the hair is divided into (*a*) internal, of two layers of large cells, the external layer consisting of transparent oval cells without nuclei, and the other layer of polyhedral

nucleated cells ; (*b*) external, of a variable number of layers of cells, becoming more columnar externally.

The hair follicle consists of an involution of the cutis vera, forming three layers : (*a*) external is very thin, made up of longitudinally arranged connective-tissue bundles, with fusiform nuclei and elastic fibres ; (*b*) middle is thicker, and made up of transverse undeveloped fibrous tissue, with rod-shaped nuclei ; (*c*) internal, of a thin, striated, transparent membrane of endothelial cells. To the outside of the follicles thin bundles of unstriped muscular fibre are attached, and into the follicle open the ducts of sebaceous (simple tubular) glands, generally one on each side.

NAIL.

Sections of a finger-nail *in situ* and of the subjacent bed or matrix should be made and double-stained.

Structure.—A nail is composed of flattened epithelial scales, and is equivalent to the superficial or horny layer of the epidermis. The deeper layers of the nail are softer than the more superficial. Underneath the nail are highly vascular papillæ which form the bed or matrix. Posteriorly it is received into a groove in the skin (root). The growth of the nail is effected by constant additions of cells to the root and under surface, so that it grows in length and in thickness at the same time.

CHAPTER XI.

GENITO-URINARY ORGANS.

(A) KIDNEY.

THE kidney should be hardened in the same way as the liver. Sections should be made in various directions. Sections of an injected kidney should also be prepared. The best injecting material is either carmine-gelatine or Berlin blue.

Structure.—There is a distinct fibro-areolar coat, or capsule, thin, firm, smooth, and easily detached. The proper substance of the organ is divided into three regions, *the cortical region*, *the boundary layer*, and *the papillary region*.

On a vertical section the cortex is that lighter part nearest the capsule, whilst the redder portion is the medulla, which is seen to be made up of a number of pyramidal portions, each papilla converging to the interior and towards branches (*calices*) of the dilated portion of the main duct or *pelvis* of the kidney. Each calyx incloses two or three papillæ. The part of the base of the pyramid towards the cortex, between it and the papillary portion, is called, as above mentioned, *the boundary layer*. The pyramid itself is called the *pyramid of Malpighi*. The papillary portion appears distinctly and vertically striated in consequence of the vertical direction of both tubules and blood-vessels, of which the kidney is principally made up. The boundary layer is also striated for a similar reason ; but the cortex, although containing vertical columns, from the arrangement of some of the tubules (*medullary rays*), no longer contains the blood-vessels arranged in vertical directions, nor are all the tubules straight,

but convoluted, forming the *labyrinth*. From the medullary rays diminishing in thickness from the boundary layer outwards towards the capsule, each presents a triangle, with its base at the boundary layer. These triangles are called the *pyramids of Ferrein*. The cortical substance separates the pyramids from each other, and incloses them everywhere except at the papillæ ; one layer of it, situated immediately beneath the capsule, forms the most superficial part of the organ. The portion of the cortical substance intervening between two pyramids is known as the *columns of Bertini*.

The papillæ are studded with minute openings leading into tubes (*tubuli uriniferi*), through which the urine passes out into a primary division (*infundibulum*) of the pelvis, or dilated part of the duct (*ureter*) of the kidney.

The tubes of the pyramids, as they pass up, divide again and again at very acute angles, until they arrive at the cortical layer, and then become convoluted. Each tube begins in a spherical dilatation (*Malpighian capsule*), enclosing a tuft of minute vessels (*Malpighian tuft*). Arising thus in the cortex, a tube is at first convoluted, and consists of a basement membrane lined and almost filled with granular epithelium ; afterwards becoming smaller, it passes straight down the pyramid towards the papilla, and returns again, forming a *looped tube of Henle* lined with squamous epithelium, then again becomes convoluted, and finally joins a branch of a straight tube of the pyramid (*collecting tube*). The collecting tubes are lined with columnar epithelium, and, joining together, form the excretory tubes or ducts of Bellini, which open at the papilla.

A renal tube is said to be made up of the following sections :

(1) *The Malpighian capsule*, lined with squamous epithelium.

(2) *The neck*, a constricted portion joining the capsule, and lined in the same way.

(3) A portion enlarged and convoluted, called the *proximal convoluted tube*, lined with polyhedral or short columnar cells, with a lumen of about one-third of the diameter of the tube. The cells are vertically striated.

(4) *The spiral tube*, which passes downwards, the structure of which is similar to the last described section.

(5) The constricted portion, called *the descending limb* of the loop of Henle, lined with squamous epithelium.

(6) *The loop of Henle,* lined with squamous epithelium.

(7) *The ascending limb*, which becomes rather suddenly enlarged, lined with striated epithelium.

(8) *The spiral portion of ascending loop* is again somewhat constricted.

(9) The ascending loop again becomes narrower, but is straight.

(10) *The irregular tubule* has a very irregular and angular outline, sometimes being three or four times as thick as at others ; this is due to the irregularity in the size of the contained epithelium. The cells are striated, angular and imbricated.

(11) *The intercalated section* (Schweigger-Seidel), or the distal convoluted tube, is similar in structure to the proximal convoluted tube.

(12) and (13) Curved collecting tubes are thin tubes, lined with polyhedral cells, or spindle-shaped and flattened.

(14) The straight collecting tube, which passes into the boundary layer, and enters—

(15) The large collecting tube, or *tube of Bellini.*

(16) The tube of Bellini, having anastomosed with similar tubes, forms the main tube of the pyramid which opens into the calyx of the pelvis, with a '*mouth*' at the apex of the papilla.

Blood-vessels.—The blood-supply of the kidney is furnished by the renal artery, which divides into branches which lie between the cortex and the boundary region ; smaller vessels pass up and enter the cortex, and pass down to supply the medulla. The vessels of the cortex pass up in the labyrinth between the medullary rays (interlobular) and give off transverse branches, the afferent vessels of the Malpighian tufts ; these break up within the capsule into convoluted capillaries, re-uniting into the efferent veins; these again break up into capillaries around the convoluted tubes, to be afterwards collected into small branches of the renal vein. The vessels of the medulla break up in the boundary region, and send off straight vessels between the tubes of the papillary region (*vasa recta*) ;

the vessels decrease in number towards the papilla, as most of them break up into capillaries around the tubules, which capillaries anastomose near the cortex with those of that region. The veins of the papillary region begin simply in the papilla, increasing in size and number as they pass upwards; join with the veins of the cortex to form the main branches of the renal vein which accompany the main branches of the renal artery, and lie between the cortex and medulla, as above-mentioned.

A certain amount of interstitial connective tissue is found supporting the tubes and blood-vessels.

(B) URETER.

To prepare the ureter for section-cutting tie one end of it, and distend it with chromic acid and spirit; leave it for one day in the same mixture; then cut it into short lengths, and remove to spirit for a week. The cells may be shown by hardening a piece of ureter in bichromate of potash 1 per cent. solution, staining deeply in logwood, and scraping the inside, teasing and mounting in glycerine.

Structure.—Consists of three coats: (1) An external *fibrous;* (2) a middle of two layers (circular and longitudinal) of unstriped *muscular fibres;* (3) an internal or *mucous*, lined by stratified epithelium, the upper cubical cells of which have their under surfaces hollowed out to receive the second layer of pear-shaped cells.

(C) BLADDER.

The bladder should be prepared in the same way as the ureter. Double staining with eosin and hæmatoxylin brings into view the differences in the form of the lining cells.

Structure.—Consists of four coats: (1) An external or *serous* —is incomplete, as it is only found at the upper and posterior parts. (2) *A muscular*, consisting of three layers more or less complete—viz., (*a*) external longitudinal, (*b*) circular, (*c*) internal longitudinal. (3) *A submucous* of connective-tissue. (4) *A mucous*, lined with stratified epithelium, the upper layer being

made up of polyhedral cells, with one, two, or three nuclei, presenting depressions with intervening ridges for the second layer of club-shaped cells ; the next layer is made up of more fusiform cells.

(D) PROSTATE.

The prostate should be immersed in a $\frac{1}{4}$ per cent. chromic acid for two days, and should then be removed to spirit.

Structure.—In structure the prostate consists of small glands imbedded in an abundance of muscular fibres and connective-tissue.

The glands consist of numerous small saccules, opening into elongated ducts, which unite into a smaller number of excretory ducts. *The acini*, in the upper part of the gland, are small and hemispherical ; whilst in the middle and lower parts the tubes are longer and more convoluted. The acini are of two kinds, (*a*) lined with a single layer of thin and long columnar cells, each with an oval nucleus in outer part of wall; (*b*) acini resembling the foregoing, but with a second layer of small cortical, polyhedral, or fusiform cells between the membrana propria and the columnar cells. *The ducts* are lined by a layer of columnar cells, beneath which is a layer of small polyhedral cells.

The tunica adventitia is formed of loose connective-tissue containing fat.

Large *blood-vessels* pass into the interior of the organ to form a broad, meshed, capillary system. *Nerve-trunks* and numerous large ganglion cells surround the cortex. Paccinian bodies are also found in the substance of the prostate.

(E) VAS DEFERENS.

The vas deferens should be prepared by hardening in a 2 per cent. bichromate of potash solution for fourteen days, after which it should be placed in spirit.

Structure.—Like the vesiculæ seminales (see p. 123) it consists of three coats : (1) *An external*, of connective-tissue, outside which longitudinal fibres of unstriated muscles are often seen.

(2) *A muscular*, two longitudinal layers with an intermediate circular one.

(3) *A mucous*, of connective-tissue and elastic fibres ; this layer is often thrown into three or four longitudinal ridges ; the epithelium consists of columnar epithelium, ciliated only near the epididymis.

The nerves form a plexus in the tunica adventitia.

(F) TESTICLE.

Place the testicles, preferably of rat or cat, after making two or three cuts in them, in equal parts of chromic acid $\frac{1}{2}$ per cent. and methylated spirit. Change three times in a week, and remove to spirit, or inject a 1 per cent. solution osmic acid into the tunica albuginea, then place in strong spirit for several days, and afterwards for two days in absolute alcohol previous to making sections. Stain with hæmatoxylin or carmine ; prepare and mount as usual.

Structure.—The outer coat consists of connective-tissue, the *tunica albuginea*, from which radiate incomplete septa uniting into a thick wedge-shaped body, the corpus Highmori.

The testicle is divided by these septa into lobes, each consisting of small and convoluted tubes, the *tubuli seminiferi*. These tubes are composed of a basement membrane of flattened endothelial cells, a single row in small animals, more than one in large animals, within which are a number of cells not arranged in any definite order—the *seminal cells*. The *outer* form a single row. The tubuli seminiferi have a uniform diameter of $\frac{1}{150}$ to $\frac{1}{200}$ inch; they commence in free closed extremities or in anastomosing arches, and unite to form the vasa recta. In transverse section the seminal tubules are seen to have a narrow lumen surrounded by polygonal cells, of which the peripheral ones are arranged radially. In the interstitial connective-tissue between the tubuli seminiferi are a number of connective-tissue corpuscles. From the seminal cells the spermatozoa are developed.

The *vasa recta*, about twenty in number, are $\frac{1}{90}$ to $\frac{1}{70}$ inch in diameter. They possess very thin walls, and pass upwards and backwards to terminate in the rete vasculosum testis.

The *rete testis* is lined with pavement epithelium, and

opens into twelve to twenty vasa efferentia, forming the coni vasculosi.

The *coni vasculosi* are $\frac{1}{50}$ inch in diameter, and open into the canal at the epididymis.

The **epididymis** and **vasa efferentia** contain plain muscular fibres; the lining cells are columnar and ciliated, elongated in the epididymis, shorter in the vasa efferentia.

Remove and draw *spermatozoa* from the fresh glands, and notice (*a*) the head, (*b*) the middle portion, (*c*) the caudal extremity.

The *blood-vessels* surround the convoluted tubules with a long-meshed wide capillary plexus.

The *lymph passages* form an extensive canalicular system.

(G) VESICULÆ SEMINALES.

The vesiculæ seminales may be prepared either in $\frac{1}{6}$ per cent. chromic acid for seven days, followed by spirit, or by hardening in methylated spirit.

Structure.—(1) There is an external *connective-tissue* coat. (2) A middle *muscular* coat of three layers, the internal of longitudinal fibres, middle of circular fibres, external of longitudinal fibres. (3) A *mucous* coat thrown into rugæ, the epithelium of cylindrical cells provided with striated borders, the deep layer being polyhedral. The mucous membrane contains a few muscular fibres.

Ganglion cells and *nerve plexuses* are numerous in the outer coat. According to Leydig, a number of racemose *glands* are present.

(H) THE PENIS.

The penis of a human fœtus should be used, if it can be obtained, otherwise that of the cat or dog. It should be injected from the abdominal aorta, after ligature of the external iliac arteries, and should then be hardened in $\frac{1}{4}$ per cent. chromic acid for a fortnight. Sections should be made in various parts.

Structure.—(*a*) *The urethra* is lined by stratified pavement *epithelium* in the lower part of the prostatic and membranous

portions ; in the upper half the epithelium is of the stratified transitional variety ; in front of the bulb the epithelium becomes columnar, whilst the fossa navicularis is again lined with stratified pavement epithelium. *The mucous membrane* consists chiefly of fibrous connective-tissue, intermixed with which are many elastic fibres. It is surrounded by muscular tissue of the unstriped variety. In the membranous portion many large veins run amongst the bundles of muscular tissue. Many *mucous glands* are present.

(*b*) *The corpora cavernosa* consists of a matrix, chiefly of unstriped muscle-fibres, intermixed with which is a little connective-tissue and a few elastic fibres. The matrix is arranged in bundles, and separates the very large *venous sinuses* which constitute the greater part of the substance of each corpus cavernosum. The sinuses anastomose with each other to form plexuses, and each is lined by a single layer of flattened endothelial plates. The arteries run in the muscular trabeculæ.

(*c*) *The corpus spongiosum urethræ* consists of an inner portion or plexus of longitudinal veins, and of an outer or really cavernous portion identical in structure with that which has just been described. *The lymphatics* of the penis are very numerous.

The nerves form a dense subepithelial plexus.

Cowper's glands resemble the sublingual gland ; they are large compound tubular mucous glands.

(I) OVARY.

The ovaries of a cat or rabbit are placed, with as little handling as possible, in a mixture of equal parts of spirit, and $\frac{1}{2}$ per cent. chromic acid solution for two or three days, and afterwards in spirit. The sections should be stained with hæmatoxylin or carmine.

Structure.—The ovary consists of an encapsuled stroma and embedded Graafian follicles.

The outer coat or capsule consists of low columnar epithelium cells, beneath which is a firm layer of fibrous tissue.

The stroma of fibrous tissue and elastic fibres containing blood-

vessels, and in the deeper portion muscular fibres. The cortical portion contains a large number of closely-set vesicles, $\frac{1}{100}$ inch in diameter. Each vesicle, or primordial ovum, is surrounded by a corona of small nucleated cells. Below this layer of vesicles are more advanced ova, the deepest being the most mature.

The Graafian follicle, $\frac{1}{20}$ to $\frac{1}{6}$ inch in diameter, contains a ripe ovum, and is surrounded by fibrous tissue, and by the tunica vasculosa, more internally by the tunica granulosa, consisting of several layers of granular prismatic cells. In a thickened portion of the tunica granulosa (discus proligerus), the ovum is embedded on the inner surface and to one side of the Graafian follicle. The tunica granulosa is separated from the discus proligerus, except at their point of union, by a space containing a clear albuminous fluid. *The ovum*, $\frac{1}{120}$ inch in diameter, consists (*a*) of an external, firm, transparent membrane, which is finely striated radially (vitelline membrane, or zona pellucida) ; (*b*) of a mass of granular protoplasm (vitellus, or yolk) ; (*c*) of a small clear vesicle, $\frac{1}{700}$ inch in diameter (germinal vesicle), embedded in the vitellus, and which encloses (*d*) a dark granular spot (germinal spot, or macula germinativa), $\frac{1}{3000}$ inch.

The corpus luteum is a Graafian follicle which has discharged its ovum ; it is filled with a reddish-yellow mass of elongated cells, the colour being due to the formation of pigment, which, however, is not derived from the slight hæmorrhage which takes place on the escape of the ovum.

(J) UTERUS.

The uterus of a cat or rabbit should be distended with a mixture of equal parts of $\frac{1}{2}$ per cent. chromic acid solution and strong spirit through the vagina. The openings into the organ should be tied, and the organ should be removed to a bottle containing same mixture. The solution should be changed at the end of twenty-four hours and the uterus laid open.

Structure.—*The external serous coat* is derived from the peritoneum.

The muscular coat is intermixed with fibro-areolar tissue

blood-vessels, lymphatics, and some veins. The muscle is arranged in three layers : (*a*) the external longitudinal, the weakest coat ; (*b*) transverse fibres forming the strongest layers ; (*c*) oblique fibres which become annular to form the sphincter uteri. The cells constituting the muscular layers are fusiform. with long tapering extremities ; the nucleus is always single.

The mucous membrane is smooth in the fundus and body of the organ ; it is raised into transverse folds in the upper portion of the cervix ; and forms papillæ in the terminal portion of the cervix. It is lined with columnar ciliated epithelium. The glands are tubular, often spiral, sometimes slightly branched. They are found in the fundus and body, and are lined with ciliated epithelium. Small closed sacs (ovula Nabothi) are also distributed regularly over the mucous membrane.

The blood-vessels are large and numerous ; *the lymphatics* form large plexuses in the peripheral layers of the pregnant uterus ; *the nerves* are medullated and non-medullated, a few ganglion cells being also present.

(K) FALLOPIAN TUBES.

Are prepared in the same way as the uterus.

Structure.—(1) *An external serous coat*, rich in vessels and in connective-tissue. (2) A longitudinal, and a thicker circular layer of unstriated *muscle.* (3) *A mucous membrane* thrown into longitudinal rugæ, and lined with columnar ciliated epithelium ; no glands are present, and, as yet, no nerves have been detected. The mucous membrane contains a layer of muscular mucosæ.

(L) MAMMARY GLANDS.

The gland, cut into small pieces, is placed in a solution of equal parts of spirit and $\frac{1}{2}$ per cent. chromic acid solution for two days, afterwards in weak and strong spirit. It should be stained in hæmatoxylin. Double staining may also be used.

Structure.—The mammary gland consists of a number of individual racemose glands united by intervening areolar tissue.

The lobes thus formed have a considerable quantity of adipose tissue between them, whilst the blood-vessels and the small medullated nerves run in the connective-tissue stroma.

The racemose glands open by means of ducts, *the lactiferous ducts*, which unite together until fifteen to twenty excretory canals are formed,

The galactophorous ducts, which converge towards the nipple. Near the nipple the galactophorous ducts become dilated to form sinuses, but they undergo constriction again before opening to the exterior.

The gland vesicles consist of a membrana propria with flattened stellate cells, lined by low columnar epithelium. The vesicles are filled with fat-globules ; and if the oil be extracted by immersion of the gland in ether, casein remains behind. The terminal vesicles are at first simple, but as the gland develops they produce buds.

The ducts consist of areolar tissue with a circular and longitudinal layer of elastic fibres ; they are lined with low cylindrical epithelium, which becomes flattened near the nipple. Near the nipple also, and beneath the areola, unstriated muscular fibres are found.

The blood-vessels form a dense capillary network around the alveoli, forming a continuous system for each lobule.

CHAPTER XII.

DUCTLESS GLANDS.

THYROID GLAND.

SHOULD be prepared by immersion of the gland for twenty-four hours in a mixture of spirit and water, then in strong spirit, till the tissue is sufficiently hard. It may also be hardened by allowing it to remain for a month in Müller's fluid, or in $\frac{1}{4}$ per cent. chromic acid for a fortnight. Should be stained in hæmatoxylin.

Structure.—A thin transparent layer of dense areolar tissue, free from fat, containing elastic fibres. This *connective-tissue framework* traverses the interior of the organ in the form of strong trabeculæ ; it incloses rounded or oblong irregular cavities, *the vesicles.* The vesicles consist of a thin hyaline membrane lined by a single row of low cylindrical cells. The cavities of the vesicles are filled with a coagulable fluid, or more frequently with a colloidal substance. The colloidal substance increases with age, and the cavities appear to coalesce.

In the interstitial connective-tissue is a round meshed *capillary plexus*, and a large number of *lymphatics. The nerves* adhere closely to the vessels.

THYMUS GLAND.

Preparation.—As for the thyroid gland. Some sections of the fresh gland should be teased in saline solution in order that the concentric corpuscles may be minutely examined.

Structure.—*A capsule* of thin areolar tissue which sends down processes dividing the gland into lobules. The outer surface of the organ is covered with a layer of flattened cells.

Each *lobe* is made up of a number of polyhedral lobules, connected by delicate areolar tissue, which are in turn composed of small *follicles*. The follicles are composed of adenoid tissue or retiform tissue, the meshes of which are filled up with lymphoid corpuscles. The follicles are therefore comparable with the spleen, tonsils, lymphatic glands, and Peyer's patches.

Scattered in the adenoid tissue are the concentric *corpuscles of Hassall*, composed of a deeply staining substance, with high refractive index. Of these there are two kinds—one, the smaller, simple ; the other, the larger, compound. *The arteries* radiate from the centre of the gland. *The lymphatics* are large. *The nerves* are very minute, the terminations have not been traced.

PITUITARY BODY.

Should be prepared in the same way as the thyroid.

Structure.—Of two lobes—*a small posterior one*, consisting of grey nerve-tissue ; *an anterior larger one*, resembling the thyroid in structure. *A canal*, lined with flattened or with ciliated epithelium, passes through the anterior lobe ; it is connected with the infundibulum. *The gland spaces* are oval, nearly round at the periphery, spherical towards the centre of the organ ; they are filled with granules and nucleated cells. The vesicles are enclosed by connective-tissue, rich in capillaries.

PINEAL GLAND.

Should be prepared by hardening in alcohol, or by maceration in Müller's fluid ; or, better still, by means of osmic acid.

Structure.—A central cavity lined with ciliated epithelium. The glandular substance is divisible into

(*a*) *An outer cortical layer*, analogous in structure to the pituitary body ; and

(*b*) *An inner central layer*, wholly nervous. The cortical layer consists of a number of closed follicles, containing (*a*) cells of

variable shape, rounded, elongated, or stellate; (*b*) fusiform cells. There is also present a gritty matter, the acervulus cerebri, consisting of round particles aggregated into small masses. The central substance consists of white and grey matter.

The blood-vessels are small, and form a very delicate capillary plexus.

SUPRA-RENAL CAPSULE.

May be hardened in bichromate of potash, 2 per cent., for a fortnight, in Müller's fluid for a month, or in osmic acid four hours; in each case complete the hardening in spirit. The glands from the guinea-pig, rabbit, and other animals should be used and compared with the human supra-renal.

Structure.—An outer *sheath of connective-tissue* sends in prolongations, and forms the framework of the gland.

The cortical portion, divided into (*a*) an external layer of closed vesicles, the zona glomerulosa. The vesicles contain a finely granular greyish substance, no fat-globules, but generally a few small cells. (*b*) A layer of cells arranged radially, the zona fasciculata. The substance of this layer is broken up into cylinders, each of which is surrounded by the connective-tissue framework. The cylinders thus produced are of three kinds—one containing an opaque, resistant, highly refracting mass (probably of a fatty nature); frequently a large number of nuclei are present; the individual cells can only be made out with difficulty. The second variety of cylinders is of a brownish colour, containing finely granular cells, in which are fat-globules. The third variety consists of grey cylinders, containing a number of cells whose nuclei are filled with a large number of fat granules. (*c*) The third layer of the cortical portion is the zona reticularis. This layer is apparently formed by the breaking up of the cylinders, the elements being dispersed and isolated. The cells are finely granular, and have no deposit of fat in their interior; but in some specimens fat may be present as well as certain large yellow granules, which may be called pigment granules.

The medullary substance consists of closed vesicles; of elements

of the cortical substance ; of numerous blood-vessels ; and of an abundance of nervous elements. The cells are poor in fat, and occasionally branched ; the nerves run through the cortical substance, and anastomose over the medullary portion.

Blood-vessels.—The cortical portion is supplied with a rich plexus of capillaries, whose meshes are polyhedral in the outer and middle zones, and more elongated in the zona fasciculata. The medulla is supplied with a very rich plexus of wide capillaries. In all parts the blood-vessels are embedded in the trabeculæ.

Lymph spaces and sinuses, best seen between the cells of the zona fasciculata, but existing in other parts, occupy the intercellular spaces and lacunæ ; the efferent lymphatics provided with valves lie in the capsule and in the connective-tissue around the central veins.

CHAPTER XIII.

LYMPHATICS.

(A) VESSELS.

(1) **Trunks.—Preparation.**—Make sections of a thoracic duct which has been hardened in bichromate of potash, and subsequently in spirit, stain with logwood, and mount in Canada balsam.

Structure.—Lymphatic trunks, such as the thoracic duct and the lymphatics leading to the mesenteric glands, have nearly the same structure as veins, and like them consist of three coats. They are provided with valves, especially at their subdivisions. The endothelial cells lining them are elongated.

(2) **Capillaries.—Preparation.**—To demonstrate the structure of lymphatic capillaries, the epithelium covering the central tendon of the diaphragm of a rabbit or guinea-pig must be pencilled off with a camel's-hair brush, the tendon should then be stained with nitrate of silver, and mounted in glycerine.

Structure.—Lymphatic capillaries consist of a single layer of sinuous endothelial cells, united together by intercellular substance so as to form a membrane.

(B) GLANDS.

Preparation.—Thin sections of a lymphatic gland which has been previously hardened in dilute spirit, Müller's fluid, or in bichromate of potash, should be stained with logwood, and shaken in a test-tube half full of water for thirty minutes or more. They are then to be prepared and mounted in balsam in the ordinary manner.

Structure.—Each lymphatic gland is surrounded by a *capsule*, which consists of connective-tissue intermingled with unstriped muscular fibres. From the capsule are given off a number of *trabeculæ*, which give support to the blood-vessels, and pass into the interior of the gland, so as to divide it into a number of compartments or alveoli, which contain the adenoid tissue or proper tissue of the gland.

The *adenoid tissue* is arranged in the form of follicles in the cortex, and of rounded cords in the medulla. Between the walls of the alveoli and the proper tissue of the gland are a number of spaces lined by endothelium—the so-called *sinuses* of a lymphatic gland. These sinuses are continuous on the one hand with the afferent vessels, and on the other with the efferent vessels.

(C) THE SPLEEN.

Preparation.—Small pieces of fresh spleen are hardened in 2 per cent. solution of bichromate of potash, and subsequently in spirit, till they are fit for making sections. Injected specimens should also be examined.

Structure.—The spleen possesses two coats, a serous and a fibrous.

The *serous coat* is derived from the peritoneum, and covers the organ almost completely.

The *fibrous coat*, or tunica propria, is composed of connective-tissue, which in some animals is intermingled with a large proportion of unstriped muscular fibres. From its inner surface processes or trabeculæ pass into the interior of the organ, and interlace freely, so as to form the *trabecular framework* of the spleen. At the hilum the capsule passes in with the blood-vessels for which it forms sheaths, which become connected with the trabeculæ above described. The interstices between these trabeculæ contain the proper tissue of the spleen, or *spleen pulp*. The spleen pulp is composed of an adenoid reticulum, forming meshes, with endothelial plates attached ; into these meshes the small arteries pour their blood, and with these meshes, by their widening out, and by the

arrangement of the endothelial plates into a distinct lining, the veins are continuous. The spleen pulp contains red blood corpuscles in all stages of decay and renovation, a large number of colourless corpuscles and blood pigment. Small arteries pass off almost at right angles from the branches within the trabeculæ, into the spleen pulp, and exchange their outer coat of connective-tissue for one of adenoid tissue. This adenoid sheath, which takes the place as it were of the lymphatic vessels which surround the arteries whilst they lie in the trabeculæ, forms cords of adenoid tissue, with arteries occupying some position, not necessarily the central. The cords are not everywhere of the same diameter, increasing here, diminishing there ; and on section appear as more or less circular masses of adenoid tissue, highly vascular when injected. To these masses seen on section the term *Malpighian corpuscles* has been applied.

CHAPTER XIV.

THE SPECIAL SENSES.

(A) THE EAR.

Preparation.—Remove the lower jaw from a recently killed guinea-pig, in order to expose the auditory bulla. Carefully break open the bulla after removing the soft parts from it, and look for the cochlea. Remove the cochlea by chipping away the surrounding bone, and immerse it at once in chromic and hydrochloric acid mixture until the bone is soft enough to be cut with ease. Place it then in weak spirit, which should be changed as often as it becomes yellow from the picric acid. Finally, put the cochlea into absolute alcohol for twenty-four hours before imbedding it. The preparation should be stained in picrocarmin, dehydrated, cleared and saturated with paraffin, and cut with a heavy microtome on a glass plate.

Structure.—*The cochlea* is a gradually tapering spiral tube, winding round a central column, *the modiolus.* It is divided along its whole extent by a spiral lamina, which projects from the modiolus, into two main portions—the scala tympani and the scala vestibuli.

The spiral lamina is partially osseous and partially membranous. The membranous portion, *the basilar membrane*, is connected to the outer wall of the cochlea by its union with the *spiral ligament*, which is a projection inwards of the periosteum and subperiosteal tissue of the cochlea.

The scala vestibuli is subdivided into scala vestibuli proper, and *ductus cochleæ* or *scala media*, by the *membrane of Reissner*, which passes from the spiral lamina to join the lining periosteum.

The membrane of Reissner is composed of a delicate membrana

propria, continuous with the periosteum covering the scala vestibuli. It is lined with a layer of flattened endothelium on the face turned towards the scala vestibuli; whilst that bounding the ductus cochleæ is provided with a single layer of polyhedral cells.

The periosteum consists of ordinary connective-tissue, thickened here and there by retiform tissue.

The spiral ligament, to which the basilar membrane is attached, consists of periosteum thickened by the retiform tissue, the cells being elongated, and radiating from the attachment of the basilar membrane. At this point there is generally a large blood-vessel; whilst between the spiral ligament and the membrane of Reissner the periosteum contains pigment-cells and a number of blood-vessels.

The floor of the ductus cochleæ is formed of a narrow portion of the spiral lamina, and of the basilar membrane. This portion terminates in a border which is C-shaped when seen in section, the lower limb of the C being prolonged and tapering. This limb is the end of the osseous lamina; it is covered by a thin membrane. The upper portion of the C is the *limbus* of the spiral lamina, whilst the bay of the C is called the *spiral groove*.

The limbus has a jagged edge, as it is raised into a number of tooth-like projections.

The organ of Corti forms a portion of the epithelium covering the basilar membrane; it consists of an outer and inner set of stiff rod-like bodies. The feet of the rods rest upon the basilar membrane, whilst they incline towards each other until they meet at their heads. By the meeting of the rods an arch is formed over the basilar membrane; it is filled with endolymph. On the inner side of the inner rods, and the outer side of the outer rods, are epithelial cells with short hair-like prolongations, the *inner and outer hair-cells;* the outer cells are more numerous and more elongated than the inner cells. The hair-like prolongations of the outer hair-cells project through rings which surround the tops of the cells, and which are bounded by minute fiddle-shaped cuticular structures—*the phalanges. A reticular membrane* is thus formed, which covers this part of the

organ of Corti. On either side of the two sets of hair-cells the epithelium passes continuously into the simple layer of cubical cells, which is found in the spiral groove, and covering the outermost part of the basilar membrane. The whole organ of Corti is also covered by a thick and highly elastic *tectorial membrane.*

The inner rods are smaller and more numerous than the outer rods ; they may be compared to the upper portion of the human ulna ; whilst *the outer rods* resemble the head and neck of a swan. The concavity of the inner rod receives the rounded portion of the outer rod, which would correspond to the back of a swan's head ; whilst the beak of the swan becomes connected to the reticular lamina. Both rods are more slender towards their middle, and expand again, so as to rest by a widened foot upon the basilar membrane ; both are longitudinally striated. In the head of the outer rod—and occasionally, also, in the inner rod—is an oval nucleus, staining more deeply than the rest of the cell.

Structure of the wall of the membranous semicircular canals.—The wall consists, from without inwards, of (*a*) *an external fibrous layer*, containing numerous nuclei, blood-vessels, and irregular pigment-cells. This layer is especially developed at the ends of the oval section where it coalesces with the ligamenta labyrinthi canaliculorum. (*b*) *The tunica propria*, which presents, after staining, a delicately striated and granular appearance. (*c*) *Capilliform processes*, which project into the interior of the canal, except at the part where the membranous canal touches the bone. (*d*) *The epithelium*, a single layer of pavement epithelium-cells investing the papillæ ; it is continued into the depressions between them.

In the ampullæ (*a*) *the fibrous layer* forms a loose meshwork, whilst (*b*) *the tunica propria* is so much thickened as to cause a rounded transverse projection into the cavity—*the crista acustica*, or *septum transversum.* (*c*) *The epithelium*, covering the crista acustica, consists (i.) of long cylindrical cells, each with a large nucleus ; these cells support the other nervous and epithelial elements, and rest upon the tunica propria ; (ii.) fusiform cells which lie between the columnar cells : each cell has a long stiff cilium, *the auditory hair*, and is in direct connection with

the ultimate fibrillæ of the auditory nerve. (*d*) *The nerves*, after passing through the tunica propria, form a very delicate plexus in the epithelial layer.

(B) THE NOSE.

Preparation.—Small pieces of the upper turbinal bones from the head of a freshly killed sheep, dog, or rabbit, should be treated in the chromic acid and hydrochloric acid solution for a week, or in $\frac{1}{4}$ per cent. bichromate of potash, or in 1 per cent. osmic acid, for forty-eight hours. Sections may be made through the nasal region of a young guinea-pig's head which has been previously hardened in chromic acid and spirit. The fresh tissue may also be treated according to the chloride of gold method.

Structure.—In a vertical section through the septum nasi, the osseous portion is seen to be invested by *periosteum*, which is immediately covered by a thick layer of elongated *tubular glands*, some simple, others more complex—the glands of Bowman. These glands have an epithelial lining of granular spherical cells at the base ; of a more polygonal and less granular form near the excretory duct. The ducts open on the surface between the elements of the external layer. The glands become less numerous and ultimately disappear at the point where the olfactory region passes into the ordinary mucous membrane, being replaced by the *mucous glands*. The glands are separated from each other by ordinary *connective-tissue*, in the deeper layers of which are *pigment-cells* and free *pigment masses*, as well as *capillaries* and ramifications of the olfactory nerves.

The epithelium, which covers the mucous membrane, consists of an external finely striated portion and an internal granular layer. In the newt the epithelium-cells may be separated into groups, by teasing after maceration for forty-eight hours in Müller's fluid. Each group consists of two kinds of cells ; one kind, which is larger than the other, presents an elongated oval form, and is situated externally. The smaller of the two kinds, or *olfactory cells*, possess a large round nucleus and two very long fine processes, of which the thicker runs outwards, whilst the finer is directed inwards. The external process is composed of

two substances, an outer, which swells up under the influence of certain reagents, and an internal thread, which remains unaffected. In man and mammalia generally the olfactory cells have no cilia. The *larger cells* are provided with oval nuclei, and extend through the whole thickness of the epithelial layer. The external portion of these cells is more or less cylindrical, and is striated longitudinally. A row of dots can be distinguished upon the external extremities. The trunks of the *olfactory nerve* run in the glandular layer either obliquely or horizontally. The ultimate fibrils of the nerve pass into the epithelial layer and probably into the olfactory cells.

For an account of the Organ of Jacobson, see Appendix.

(C) THE OPTIC APPARATUS.

(1) **The Eyelids.**—*Preparation.*—The eyelids and the lachrymal apparatus may be obtained from a pig if human material is not available. They should be placed in the usual solution of chromic acid and spirit.

The skin of the eyelids consists of an epidermis of flattened cells and of a thin corium ; the papillæ of the latter are small, and the subcutaneous tissue very loose, containing numerous and wide lymphatics ; a few groups of fat-cells are present. The sweat-glands are small, and the hairs fine, with small sebaceous glands. At the anterior edge of the free margin of the lids the papillæ become larger, and the hairs are converted into eyelashes. Immediately behind the cilia are the ducts of *the glands of Mohl*, which frequently open into the ducts of the sebaceous glands. The glands of Mohl closely resemble in structure the sweat-glands.

Next to the subcutaneous tissue are bundles of striated fibres of *the orbicularis muscle*, separated from each other by loose connective-tissue, which occasionally contains fat-cells.

The tarsal plate is a dense felted mass of fibrous tissue which does not contain any cartilage : its anterior and posterior surfaces are intimately connected by bundles of connective-tissue with the skin of the eyelid and with the conjunctiva.

Near the posterior edge of the free margin are the mouths of *the Meibomian glands*, arranged in a single row. The ducts are embedded in the tarsal plate, and are in direct connection with

saccular single or branched alveoli ; the glands resemble sebaceous glands. At the posterior edge of the lid the stratum Malpighii becomes modified, and passes into the conjunctiva. A comparatively thick layer of muscle, the *musculus ciliaris Riolani*, intervenes between the mouths of the Meibomian glands and the eyelashes.

The mucosa of the conjunctiva palpebræ is a comparatively thick connective-tissue membrane, which generally contains a variable amount of diffuse adenoid tissue.

The conjunctiva palpebræ itself consists of one or two layers of small polyhedral cells, upon which is superposed a layer of longer or shorter conical or columnar cells, amongst which are frequently seen some goblet-cells. Small *mucous glands* are embedded in the tarsal plate in the neighbourhood of the conjunctiva palpebræ.

(2) **The lachrymal gland** is a serous gland ; it is divided into lobes and lobules by the connective-tissue capsules. The larger *interlobular ducts* are lined with a layer of thin columnar epithelium cells.

The intralobular ducts are also lined with columnar cells, whose external portion is distinctly fibrillated, whilst the inner portion is only slightly striated : the nucleus being situated about the centre of each cell. The intermediate portions of the intralobular ducts, and the parts immediately opening into the alveoli, consist of fine tubes lined with a layer of flattened cell-plates, which are often imbricated.

The alveoli are more or less tubular, and are provided with lateral and terminal tubular or saccular branches.

The membrana propria consists of branched flattened homogeneous cells, from which septa extend between the cells of the glandular epithelium.

The glandular epithelial cells form a single layer of polyhedral, or cubical, granular-looking cells, each provided with a spherical nucleus. The distribution of *blood-vessels* is identical with that of the salivary glands.

(3) **Cornea.**—The anterior part of a human eye which has been hardened in 2 per cent. solution of bichromate of potash for a fortnight, and then in spirit, should be used.

To demonstrate the connective-tissue cells and nerves, the fresh cornea of a frog or rabbit should be cut out, and placed for about an hour and a half in chloride of gold solution $\frac{1}{2}$ per cent., then in slightly acidulated water, and exposed to the light for twenty-four hours or more, according to its intensity, and mounted whole (or in sections in the case of the rabbit's cornea) in glycerine.

In order to stain the ground substance, the fresh or living cornea of a pithed frog should be pencilled with solid nitrate of silver, well washed in distilled water and mounted in glycerine.

Structure.—The cornea consists of five layers : (1) The superficial or conjunctival layer is composed of stratified *epithelium*, of three or four distinct strata of nucleated cells, the lowest of which is columnar.

(2) This layer merges into a thin homogeneous layer (distinct only in the human eye), the *anterior elastic lamina*, which does not differ from the substance of the cornea proper, except in its greater density and in the absence of corneal corpuscles.

(3) The *proper substance* of the cornea, made up of alternating layers of fibrous tissue parallel to the surface. These are separated from one another by the ground substance, in which are the cell-spaces, of irregular branched form, which freely communicate with the cell-spaces of the same, as well as of other layers. In the chloride of gold cornea, these cells appear as large and branched granular dark-red or black cells with large oblong nuclei containing nucleoli. In the spaces, but not filling them up entirely, are the corneal corpuscles, which are branched cells of various forms.

(4) Membrane of Descemet, or *posterior elastic lamina*—a firm, structureless, but brittle, transparent membrane covered by

(5) A layer of endothelial or *epithelial cells*.

There are no *blood-vessels* in a healthy cornea, except at the periphery.

Nerves enter the proper substance of the cornea, and, becoming transparent, form a plexus, from which finer branches going forward form another ' sub-epithelial plexus,' from which, again,

finer fibrils pass among epithelial cells, forming the 'intra-epithelial plexus.'

(4) **Retina.**—The posterior part of the eye of a pig (if no fresh human eye can be had) is hardened in Müller's fluid for a week, and then transferred to alcohol; pieces of the retina may then be stained in logwood, and cut. Double-staining with eosin and aniline green, or with aniline rose and aniline green, helps to differentiate the layers.

Another method is to place in 2 per cent. solution of osmic acid for four hours, then in water for one hour, to get rid of the excess of osmic acid, and finally in logwood. The retina, thus treated, should be embedded in cacao-butter, instead of the ordinary wax.

Structure.—It consists of eight layers in the following order, from within outwards:

1. *A layer of nerve fibres*, which is wanting at the yellow spot: the fibres consist of axis cylinders only; it diminishes in thickness anteriorly.

2. *A layer of nerve-cells* (ganglionic layer), consisting of cells of a spheroidal or pyriform figure; one process of each extends into the first layer, and is doubtless continuous with it. From the other end of the cell, one or more processes extend outward for a variable distance into the next layer. In the yellow spot there are several layers of cells; elsewhere, only one layer.

3. *An inner molecular layer*, a dense network of various fibrils which appear as a thick stratum of granular-looking substance.

4. *An inner nuclear layer* consists of transparent nucleus-like bodies, of at least four kinds—(*a*) a few connected with the fibres of Müller (to be described below); (*b*) the largest number, like bipolar cells, one pole unbranched, passing inwards, and being connected with a nerve-fibre—the other, thicker and branched, running outwards, is supposed to break up into a plexus in the outer molecular layer; (*c*) unbranched cells found as a complete stratum at the innermost part; (*d*) scattered in the outermost part, are rounded cells, of large size, with only one process.

5. *An outer molecular layer*, thinner than (3), but otherwise of the same structure.

6. *An outer nuclear layer*, consists roughly of two kinds of corpuscles embedded in a reticular matrix : (*a*) those connected with the rods are most numerous, and may be considered as dilatations situated in the centre of the fine rod fibres, they have an elliptical striated nucleus, but no nucleolus ; (*b*) those connected with the cones are fewer, pear-shaped, not striated, and situated nearer the outer part of the layer in the thicker cone-fibre.

7. *A layer of rods and cones*, is composed of elliptical elongated bodies, *the rods*, and shorter, thicker, club-like bodies, *the cones ;* each consists of two parts, inner and outer, of which the outer is transversely striated and smaller, and in the cones tapers to a point, whilst the inner is fibrillated externally, but homogeneous internally.

8. *The pigmentary layer*, or *uvea*, consists of a single stratum of hexagonal pigment-cells.

The fibres of Müller consist of bands, which pass through all the layers of the retina, binding them together ; they commence by a broad base, forming by their union the *membrana limitans interna ;* and, at the outside of the retina, the *membrana limitans externa.* In the inner nuclear layer they give off processes which contain a clear, oval nucleus. In the outer nuclear layer they break up into fibrils, and partially enclose the rod and cone fibres.

Blood-vessels.—The arterial and venous branches are situated internally under the layer of nerve-fibres. The capillaries are arranged in plexuses, with large meshes. They occur in the inner molecular layer, one plexus being situated near the inner nuclear layer, the other near the layer of nerve-cells. A plexus also exists in the inner nuclear layer, whilst another lies more superficially in the outer molecular layer.

(5) **Crystalline Lens.**—(*a*) Harden the eye of a frog, from which the cornea has been removed, in a solution consisting of one part of fuming nitric acid, three parts of water, and one part of glycerine. At the end of twenty-four hours remove it from this solution, and allow it to remain for a day in water. Tease a

portion of the lens thus prepared in glycerine, and mount it in Farrant's solution or glycerine.

(*b*) Sections of the lens should be made from eyes which have been hardened for a fortnight in Müller's fluid, and afterwards in weak spirit.

(*c*) The capsular epithelium is best demonstrated by staining the uninjured lens of the frog in nitrate of silver, and afterwards mounting portions of the anterior capsule in glycerine.

Structure.—(*a*) *Of the capsule.* The portion which covers the anterior surface of the lens consists of a thick elastic layer, immediately behind which is a single layer of granular hexagonal epithelium cells, each of which is provided with an oval nucleus. The elastic lamina covering the back of the lens has no such lining epithelium, but is in close contact with the lens-fibres.

(*b*) *The lens* itself is composed of (1) *The lens-fibres*, which are elongated bands running from the posterior to the anterior surface ; they are broader behind than in front. Each fibre contains a nucleus, which is more distinct in the peripheral than in the central fibres. Every fibre is hexagonal when seen in transverse sections, and is serrated along its narrow edge, the teeth of one fibre fitting into the notches of its neighbour. (2) *The interstitial substance* is like that of connective-tissue, it is permeated by lymph channels.

(6) **Iris.**—The iris from an eye hardened in chromic acid and spirit mixture, together with the ciliary processes, can be cut by means of the freezing microtome ; the operation requires much care. The whole iris of a small animal may be mounted and examined in a recent state in saline solution.

Structure.—The iris is principally made up of connective-tissue and blood-vessels. This forms the middle layer. In front it is covered by *endothelium*, which may contain pigment, a homogeneous basement membrane intervening. Behind there are similar layers, *i.e.*, an endothelial pigmentary layer, *the uvea*, and an intervening basement membrane. Around the inner border is a circular muscle of unstriped fibres, the *sphincter pupillæ*. Under the uvea, *i.e.*, between that and the iris proper,

is a thin radiating membrane of muscle cells, which passes outwards from the sphincter. This is the *dilator pupillæ*. The *blood-vessels* are arranged in dense capillary plexuses in the tissue proper and on the sphincter. *The Nerves* form a plexus near the outer edge, from which pass off medullated fibres, terminating in the dilator and in the anterior surface of the iris proper, and also non-medullated nerves to the sphincter.

7. **Ciliary Processes.**—The ciliary processes resemble the iris in structure, having a similar connective-tissue basis, containing branched pigment cells, covered by a transparent membrane, the *lamina vitrea*, whilst external to this is the uvea, differing in no way from the uvea of the retina.

The uvea is covered by a single layer of transparent columnar cells.

The ciliary muscle is connected with the outer part of the ciliary processes, and is made up principally of fibres radiating outwards, but partly of circular bundles.

8. **Choroid.**—The choroid consists of the following coats :

(*a*) *Lamina fusca*, a loose connective-tissue, with corpuscles, both with and without pigment, branched or unbranched ; the lamina suprachoroidea is a continuation of the lamina fusca.

(*b*) *Stratum vasculosum*, in which are the large blood-vessels embedded in loose connective-tissue.

(*c*) *An elastic layer* containing small arteries and veins, covered on each side by endothelium.

(*d*) *Membrana chorio-capillaris*, which contains the dense capillary meshwork, covered by cells, spindle-shaped and flattened, with or without pigment.

(*e*) *Lamina vitrea* (as above).

(*f*) *Uvea*, or retinal pigment.

9. **Sclerotic.**—This coat is made up of dense fibrous tissue, the bundles of which in part cross and interlace. Between the bundles are connective-tissue corpuscles, almost precisely similar to those of the cornea. These cells are contained in spaces which form an anastomosing lymph-canalicular system. Non-medullated nerve fibres are said to exist in a dense plexus in the

tissue. Near the ligamentum pectinatum iridis, at the corneo-scleral junction, is a circular canal, lined with endothelium ; it communicates indirectly with the lymph spaces mentioned above, and is called the canal of Schlemm.

PART II.

PHYSIOLOGICAL CHEMISTRY.

Apparatus and chief Reagents required.

Test-tubes.
Test-tube stand.
Retort stand.
Platinum foil and wire.
Three Berlin dishes.
Three beakers.
Sand bath.
Filter papers and funnels.
Sulphuric, Nitric, Hydrochloric, Acetic, Tannic, Carbolic, Picric and Boracic acids.

Solutions :

Magnesium chloride and sulphate.
Copper sulphate.
Sodium carbonate, sodium chloride, 10 per cent.
Caustic potash, or soda.
Ammonia and ammonium sulphide.
*Millon's reagent. (Mixed mercury nitrate and nitrite?)
Potassium ferrocyanide.
Potassio-mercuric iodide.
Mercuric nitrate and chloride, mercurous nitrate.
Lead acetate.
Calcium chloride.
Litmus, etc., etc.

Alcohol.
Ether.
Ozonic ether.
Lime water.
Tinc. Guaiaci.
Solid sodium chloride and magnesium sulphate.

This division of the book treats of the chief substances found in the animal body, also of foods, and the action of the digestive juices upon them, of the secretions and excretions, and of calculi. For the sake of convenience the substances are treated of in the following order : (1) Proteids, or albuminous substances, which occur in the animal tissues and in food ; Nitrogenous bodies, other than proteids : (2) Carbo-hydrates ; (3) Oil and fats ; (4) Healthy urine, and its constituents ; (5) Blood, Milk and Bile : Gall-stones ; (6) Digestive fluids ; (7) Abnormal urine and Calculi ; (8) Examination of organic substances.

* *Preparation of Millon's Reagent.*—Take equal parts by weight of pure mercury and of nitric acid, add the acid to the mercury, place in a ventilated cupboard, and leave until the mercury is dissolved—if necessary, warming slightly. Then add twice its bulk of water. After a time a crystalline white precipitate falls, and the supernatant fluid is decanted.

CHAPTER I.

PROTEIDS AND THEIR ALLIES.

PROTEIDS or albumins are bodies which are found to be present in all protoplasm, of which, indeed, they constitute the chief part. They are highly nitrogenous substances, and contain the elements carbon, hydrogen, oxygen, nitrogen and sulphur in certain proportions, which may vary (C, from 51·5 to 54·5 ; H, 6·9 to 7·3 ; O, 20·9 to 23·5 ; N, 15·2 to 17 ; and S, ·3 to 2) slightly. Associated with proteids in protoplasm are found carbo-hydrates and fatty bodies, as well as nitrogenous organic bodies other than proteids, and some salts—*e.g.*, sodium and potassium chlorides. According to some the salts are in part contained in the proteid molecule.

General Properties of Proteids.—They are all amorphous ; some are soluble, some insoluble in water ; some are soluble in saline solutions, some insoluble ; all are soluble, often with decomposition, in strong acids and alkalies ; are insoluble in strong alcohol. Their solutions, for the most part, will not pass through animal membranes, and exercise a left-handed action on polarised light.

General Reactions of Proteids.—The solutions of proteids, even if dilute, give the following tests :

(i.) *They turn yellow on heating with strong nitric acid ; the colour deepens on addition of ammonia* (xanthoproteic reaction).

(ii.) *They give with Millon's reagent a pink precipitate or mere colouration, either directly or on boiling.*

(iii.) *They give with an excess of caustic soda or potash, and a drop of copper sulphate, a violet colouration* (biuret reaction).

Many of the proteids give, in addition, the following tests :

(iv.) With excess of acetic acid, and potassium ferrocyanide, a white precipitate.

(v.) With excess of acetic acid and a saturated solution of sodium sulphate, on boiling, a white precipitate. This test is used to get rid of all traces of proteids, except peptones, from solution.

(vi.) Boiled with strong hydrochloric acid, a violet red.

(vii.) With cane sugar and strong sulphuric acid a purplish-violet.

(viii.) They are precipitated on addition of

Citric or acetic acid, and picric acid ; or,
„ „ and sodium tungstate ; or,
„ „ and potassio-mercuric iodide.

Varieties of Proteids.—Proteids are divided into seven classes, chiefly on the basis of their solubilities in various reagents. Each class, however, if it contain more than one substance, may often be distinguished by other properties common to its members.

(1) *Native-Albumins.*—Soluble in water and in saline solutions, coagulated, *i.e.*, turned into coagulated proteid, on heating.

(2) *Derived-Albumins.*—Soluble in acids or alkalies, insoluble in saline solutions and in water, not coagulated on heating.

(3) *Globulins.*—Soluble in strong or in weak saline solutions, insoluble in water, soluble in dilute acids and alkalies, coagulated on heating.

(4) *Fibrin.*—Insoluble in water, dilute saline solutions, or dilute acids or alkalies ; soluble in strong saline solutions (partly) and in strong acids ; soluble in gastric or pancreatic fluids.

(5) *Peptones.*—Soluble in water, saline solutions, acids, or alkalies ; not coagulated on heating.

(6) *Coagulated Proteids.*—Soluble only in gastric or pancreatic fluids, forming peptones.

(7) *Amyloid substance, or Lardacein.*—Generally insoluble, even in gastric or pancreatic fluids at ordinary temperatures. Gives a brown colouration with iodine.

Class I.

NATIVE-ALBUMINS.

(A) *Egg-Albumin.*
(B) *Serum-Albumin.*

(A) **Egg-Albumin :**

Preparation.—The white of an egg is cut up into pieces with a pair of scissors, shaken up with four or five times its bulk of water in a flask, and filtered through muslin.

Properties.—The solution is a transparent, frothy, yellowish fluid, neutral or slightly alkaline in reaction.

Tests.—With the solution show the following tests :

(i.) It gives all of the general proteid reactions.

(ii.) Evaporate a portion to dryness in a water bath at a temperature not exceeding 40° C. The albumen is dried up into a yellowish, transparent, glassy mass, soluble in water.

(iii.) Heat another portion to a temperature of 70°C. It is coagulated, *i.e.*, changed into a new substance, coagulated proteid, which is quite insoluble in water.

(iv.) Show that *coagulation* also takes place :

(*a*) By the prolonged action of alcohol ;
(*b*) By strong mineral acids, especially by nitric acid, also by tannic acid, or carbolic acid ;
(*c*) By ether the coagulum is soluble in caustic soda.

(v.) Show that it is *precipitated*, *i.e.*, forms an insoluble compound with the reagent, soluble on removal of the salt by dialysis, without coagulation, with either mercuric chloride, lead acetate, copper sulphate or silver nitrate, the precipitate being soluble in slight excess of the reagent.

(vi.) Show also *Heller's test*, which is a delicate one for mere traces of the albumin in solution. Pour a little strong nitric acid into a test-tube, and add gradually a dilute solution of albumin ;

the albumin is precipitated at the point of contact with the acid in the form of a fine white or yellow ring.

(B) **Serum-Albumin:**

Preparation.—Blood serum is diluted with water after the paraglobulin has been removed.

Tests.—With the solution show that

(i.) It gives similar reactions to egg-albumin, but differs from it in not being coagulated by ether.

(ii.) It also differs from egg-albumin in not being easily precipitated by hydrochloric acid, and in the precipitate being easily soluble in excess of this acid. Serum-albumin, either in the coagulated or precipitated form, is more soluble in excess of strong acid than egg-albumin.

DIALYSIS.

Native-albumins are very slightly diffusible. Show this by taking a solution of egg- or serum-albumin and placing it in a dialyser, and testing the outside liquid from time to time for albumen.

Salt should be mixed with solution of albumen, and tested for in the outside liquid. It is speedily detected ; not so the albumen.

CLASS II.

DERIVED-ALBUMINS.

(A) *Acid-Albumin.*
(B) *Alkali-Albumin.*
(C) *Casein.*

(A) **Acid-Albumin:**

Preparation.—Acid-albumin is made by adding small quantities of dilute acid (of which the best is hydrochloric, ·4 per cent. to 1 per cent.), to either egg- or serum-albumin diluted five to ten times, in a flask, and keeping the solution at a temperature not higher than 50° C for not less than half an hour.

Acid-albumin may also be made by dissolving coagulated native-albumin in strong acid, or by dissolving any of the globulins in acids.

Tests.—With a solution of acid-albumin show that

(i.) It is not coagulated on heating, but that on *exactly* neutralising the solution, a flocculent precipitate is produced. This may be done by adding to the acid-albumin solution a little aqueous solution of litmus, and then dropping a weak solution of caustic potash from a burette, until the red colour disappears. The precipitate is the derived-albumin. It is soluble in dilute acid, dilute alkalies and dilute solutions of alkaline carbonates.

(ii.) It gives the proteid tests.

(iii.) It is coagulated by strong acids, *e.g.*, nitric acid, and by strong alcohol.

(iv.) It is insoluble in distilled water, and in neutral saline solutions.

(v.) It is precipitated by saturation with sodium chloride.

(vi.) There is partial coagulation on boiling in lime-water, and further precipitation on addition to boiled solution of calcium chloride, magnesium sulphate, or sodium chloride.

(B) **Alkali-Albumin:**

Preparation.—If solutions of native-albumin, or coagulated or other proteid, be treated with dilute or strong fixed alkali, alkali-albumin is produced.

Solid alkali-albumin may be prepared by adding caustic soda or potash, drop by drop, to undiluted egg-albumin, until the whole forms a jelly. This jelly is soluble in dilute alkalies on boiling.

Tests.—A solution of alkali-albumin gives the tests corresponding to those of acid-albumin. It is not coagulated on heating. It is thrown down on neutralising its solution, except in the presence of alkaline phosphates, in which case the solution must be distinctly acid before a precipitate falls.

To differentiate between Acid- and Alkali-Albumin, the following method is useful:—

(1) Alkali-albumin is *not* precipitated on *exact* neutralisation, if sodium phosphate has been previously added.

(2) Acid-albumin is precipitated on exact neutralisation whether or not sodium phosphate has been previously added.

(C) **Casein**:

Preparation.—Casein may be prepared from milk by the

following process: Dilute the milk with three to four times its volume of water, add sufficient dilute acetic acid to render the solution distinctly acid, but not more than a few drops, and filter off the casein. To purify it, wash with alcohol and then with ether.

Casein may also be prepared by adding to milk an excess of crystallized magnesium sulphate or sodium chloride, which causes it to separate out.

Tests.—Casein gives much the same tests as alkali-albumin.

It is soluble in dilute acid or alkalies.

It is reprecipitated on neutralisation, but if potassium phosphate be present, the solution must be distinctly acid before the casein is deposited.

Class III.

GLOBULINS.

(A) *Globulin.*
(B) *Myosin.*
(C) *Paraglobulin.*
(D) *Fibrinogen.*
(E) *Vitellin.*
(F) *Globin.*

General Properties of Globulins.—They give the general proteid tests.

Are insoluble in water.

Are soluble in dilute saline solutions.

Are soluble in acids and alkalies forming the corresponding derived-albumin.

Most of them are precipitated from their solutions by saturation with solid sodium chloride.

Are coagulated on heating.

(A) **Globulin** or **Crystallin**:

Preparation.—It is obtained from the crystalline lens by rubbing it up with powdered glass, extracting with water or dilute saline solution, and by passing through the extract a stream of carbon dioxide.

Tests.—It differs from other globulins, except vitellin, in not being precipitated by saturation with sodium chloride.

(B) **Myosin**:

Preparation.—Myosin may be prepared from dead muscle by

removing all fat, tendon, etc., and washing repeatedly in water, until the washing contains no trace of proteids, mincing it and then treating with 10 per cent. solution of sodium chloride, which will dissolve a large portion into a viscid fluid, which filters with difficulty. If the viscid filtrate be dropped little by little into a large quantity of distilled water, a white flocculent precipitate of myosin will occur.

Tests.—With a solution of myosin show that

(i.) It is soluble in 10 per cent. saline solution.

(ii.) It is coagulated at 60° C. into coagulated proteid.

(iii) It is soluble without change in very dilute acids.

(iv.) It is precipitated by picric acid, the precipitate being re-dissolved on boiling.

(v.) It may give blue colour with ozonic ether and tincture of guaiacum.

(c) **Paraglobulin :**

Preparation.—Paraglobulin may be precipitated by saturating serum with solid sodium chloride or magnesium sulphate, as a bulky flocculent substance, which can be removed by filtration after standing for some time.

It may also be prepared by diluting blood serum with ten vols. of water, and passing carbonic acid gas rapidly through it. The fine precipitate is collected on filter, and washed with water containing carbonic acid gas.

Tests.—It is very soluble in dilute saline solutions, from which it is precipitated by carbonic acid gas and dilute acids.

Its solution is coagulated at 70° C.

Even dilute acids and alkalies convert it into acid- or alkali-albumin.

(d) **Fibrinogen :**

Preparation.—Fibrinogen is prepared from hydrocele or other serous transudation by methods similar to those employed in preparing paraglobulin from serum.

Tests.—Its general reactions are similar to those of paraglobulin.

Its solution is coagulated at 52°-55° C.

Its characteristic property is that, under certain conditions, it forms fibrin.

(E) **Vitellin :**

Preparation.—Vitellin is prepared from yolk of egg by washing with ether until all the yellow matter has been removed. The residue is dissolved in 10 per cent. saline solution, filtered, and poured into a large quantity of distilled water. The precipitate which falls is impure vitellin.

Tests.—It gives the same tests as myosin, but is not precipitated on saturation with sodium chloride.

It coagulates between 70° and 80° C.

(F) **Globin :**

Is the proteid residue of hæmoglobin.

Class IV.

FIBRIN.

Fibrin :

Preparation.—Fibrin can be obtained as a soft, white, fibrous, and very elastic substance by whipping blood with a bundle of twigs, and washing in a stream of water until all the adhering blood-colouring matter be removed.

Tests.—It differs from all other proteids, in having a filamentous structure. Examine with the microscope.

It is insoluble in water and dilute saline solutions, slightly soluble in concentrated saline solutions, soluble on boiling in strong acids and alkalies.

On boiling it is converted into coagulated proteid.

When dissolved in strong saline solution it gives many of the same reactions as myosin. When dissolved in acids or alkalies, is converted into corresponding derived-albumin.

It gives a blue colour with tincture of guaiacum and ozonic ether.

Class V.

PEPTONE.

Peptone :

Preparation.—Peptone is formed by the action of the digestive ferments, *pepsin* or *trypsin*, on other proteids, and on gelatine (see pp. 185, 186).

Tests.—Make a solution of commercial peptone in warm water, filter, and with the filtrate show the following tests :

(i.) It is soluble in water and in dilute saline solutions.

(ii.) It is not coagulated on heating.

(iii.) It is not precipitated by saturation with NaCl, or $MgSO_4$, or by CO_2.

(iv.) It is not precipitated by boiling with sodium sulphate and acetic acid.

(v.) It is not precipitated by addition of dilute acid or alkali.

(vi.) It is precipitated from neutral or slightly acid solutions by :

Mercuric chloride, the precipitate being only partly soluble in excess.

Argentic nitrate.

Lead acetate.

Potassio-mercuric iodide.

Bile salts.

Phosphoro-molybdic acid.

Tannin, the precipitate soluble in dilute acid, not in excess.

Picric acid (saturated solution), precipitate disappears on heating and partly returns on cooling.

Absolute alcohol (*not* with dilute). N.B. It is not *coagulated.*

Ether.

(vii.) The solution gives :

The *Xanthoproteic reaction* easily, but there is very slight, if any previous precipitation with the nitric acid.

The *Biuret reaction*—but the colour is *pink* instead of *violet* (use a mere trace of copper sulphate).

With *Millon's* test—not so easily as with native albumins.

With *Ferrocyanide and acetic acid*—only in cases where the peptone is impure (Meissner's A and B peptones), is there any precipitate.

(viii.) It *dialyses freely.*—Show this with a simple gut dialyser in the manner indicated p. 151.

Class VI.

COAGULATED PROTEIDS.

Coagulated proteids are formed by the action of heat upon other proteids ; the temperature necessary in each case varying in the manner previously indicated. They may also be produced by the prolonged action of alcohol upon proteids.

Tests.—They are soluble in strong acids or alkalies; slightly so in dilute.

Are soluble in digestive fluids (gastric and pancreatic).

Are insoluble in saline solution.

Class VII.

LARDACEIN OR AMYLOID SUBSTANCE.

Lardacein is found in organs which are the seat of amyloid degeneration.

Tests.—Insoluble in dilute acids and in gastric juice at the temperature of the body.—Coloured brown by iodine and bluish-purple by methyl violet.

THE GELATINS OR NITROGENOUS BODIES OTHER THAN PROTEIDS.

(A) *Gelatin.*
(B) *Mucin.*
(C) *Elastin.*
(D) *Chondrin.*
(E) *Keratin.*

(A) Gelatin :

Preparation.—Gelatin is contained in bone, teeth, fibrous connective-tissue, tendons, ligaments, etc. It may be obtained by prolonged action of boiling water in a Papin's digester or of dilute acetic acid at a low temperature (15° C.).

Properties.—The percentage composition is O, 23·21, H, 7·15, N, 18·32, C, 50·76, S, 0·56. It contains more nitrogen and less carbon than proteids.

It is amorphous, and transparent when dried. It does not dialyse.

It is insoluble in cold water, but swells up to about six times its volume : it dissolves readily on the addition of very dilute acids or alkalies.

It is soluble in hot water, and forms a jelly on cooling, even when only 1 per cent. of gelatin is present. Prolonged boiling in dilute acids, or in water alone, destroys this power of forming a jelly on cooling.

Tests.—A fairly strong solution of gelatin—2 per cent. to 4 per cent.—gives the following reactions :

(A) *With proteid tests:*

(i.) *Xanthoproteic test.*—A yellow colour with no previous precipitate with nitric acid, becoming darker on the addition of ammonia.

(ii.) *Biuret test.*—A violet colour.

(iii.) *Millon's test.*—A pink precipitate.

(iv.) *Potassium ferrocyanide and acetic acid.*—No reaction.

(v.) Boiling with sodium sulphate and acetic acid.—No reaction.

(B) *Special reactions:*

(i.) No precipitate with acetic acid.

(ii.) No precipitate with hydrochloric acid.

(iii.) A white precipitate with tannic acid, not soluble in excess or in dilute acetic acid.

(iv.) A white precipitate with mercuric chloride, unaltered by excess of the reagent.

(v.) A white precipitate with alcohol.

(vi.) A yellowish-white precipitate with picric acid, dissolved on heating and reappearing on cooling.

Bone consists of an organised matrix of connective-tissue which yields gelatin and inorganic salts.

Inorganic salts can be removed by digesting bone in hydrochloric acid. The gelatinous matter left retains the form of the bone. By long boiling in water it is converted into a solution of a gelatin.

When bone is heated, the first action is to decompose the organic matter, leaving a deposit of carbon. On further ignition in air this carbon burns away, and only inorganic salts (principally calcic phosphate) are left.

Take two pieces of bone, calcine one piece, and boil another in dilute hydrochloric acid in a test tube; test the ash from the one for phosphates, and test the undissolved part of the other and also the solution in which it has been boiled for gelatin.

(B) **Mucin**:

Preparation.—Mucin is the characteristic component of mucus; it is contained in fœtal connective-tissue, tendons, and salivary glands.

It may be prepared from ox-gall, by acidulation with acetic

acid and subsequent filtration, or from ox-gall by precipitation with alcohol, afterwards dissolving in water, and again precipitating by means of acetic acid.

It can also be obtained from mucus by diluting it with water, filtering, treating the insoluble portion with weak caustic alkali, and precipitating the mucus with acetic acid.

Properties.—Mucin has a ropy consistency.

It is precipitated by alcohol and by mineral acids, but dissolved by excess of the latter.

It is dissolved by alkalies and in lime water.

It gives the proteid reaction with Millon's reagent and nitric acid, but not with copper sulphate.

Neither mercuric chloride nor tannic acid gives a precipitate with it (?).

It does not dialyse.

(c) **Elastin** is found in elastic tissue, in the ligamenta subflava, ligamentum nuchæ, etc.

Preparation.—Take the fresh ligamentum nuchæ of an ox, cut in pieces, and boil in alcohol and ether to remove the fat. Remove the gelatin by boiling for some hours in water. Boil the residue with acetic acid for some time, and remove the acid by boiling in water, then boil with caustic soda until it begins to swell. Remove the alkali, and leave it in cold hydrochloric acid for twenty-four hours, and afterwards wash with water.

Properties.—It is insoluble, but swells up both in cold and hot water. Is soluble in strong caustic soda.

It is precipitated by tannic acid; does not gelatinize. Gives the proteid reactions with strong nitric acid and ammonia, and imperfectly with Millon's reagent.

Yields leucin on boiling with strong sulphuric acid.

(d) **Chondrin** is found in cartilage.

Preparation.—By boiling small pieces of cartilage for several hours, and filtering. The opalescent filtrate will form a jelly on cooling. Chondrin is precipitated from the warm filtrate on addition of acetic acid.

Properties.—It is soluble in hot water, and in solutions of neutral salts, *e.g.*, sulphate of sodium, in dilute mineral acids, caustic potash, and soda. Insoluble in cold water, alcohol, and ether. It is precipitated from its solutions by dilute mineral acids (excess re-dissolves it), by alum, by lead acetate, by silver nitrate, and by chlorine water. On boiling with strong hydrochloric acid, yields grape-sugar and certain nitrogenous substances. Prolonged boiling in dilute acids, or in water, destroys its power of forming a jelly on cooling.

(e) **Keratin.**

CHAPTER II.

CARBO-HYDRATES.

CARBO-HYDRATES are bodies composed of six or twelve atoms of carbon with hydrogen and oxygen, the two latter elements being in the proportion to form water.

They are arranged into three classes; the members of each class with which we have to do are printed in ordinary type, the most important of the others in italics.

Amyloses.	Saccharoses.	Glucoses.
$C_6H_{10}O_5$	$C_{12}H_{22}O_{11}$	$C_6H_{12}O_6$
Starch	*Saccharose, or cane sugar*	Dextrose or grape sugar
Dextrin	Lactose	Lævulose or fruit sugar
Glycogen	*Maltose*	Inosite
Inulin	*Mellitose*	*Mannitose*
Cellulose		
Gum		

(A) **Starch** ($C_6H_{10}O_5$) is contained in nearly *all plants :*

Obtain some starch from a *potato*, by grating it into a bowl of water, allow it to settle, and pour off the supernatant water ; a layer of starch remains at the bottom ; wash several times, each time allowing the starch to settle and pouring away the water ; when clean, dry at a moderate temperature.

Or from *flour*—tie in a bag and wash into a vessel under the tap ; allow to settle and proceed as above.

(Or it may be obtained from many *seeds*, *roots*, *stems*, and *some fruits*, by somewhat similar treatment.)

Characters.—It is a soft white powder composed of granules having an organised structure, consisting of *granulose* (soluble in water) and *cellulose* (insoluble in water) ; their shape and size vary according to the source whence the starch has been obtained.

Examine starch from potato, rice, and flour, under the microscope, and compare the size and shape of the granules.

Tests.—It is insoluble in cold water, in alcohol, and in ether.

It is soluble after boiling for some time, and may be filtered (this is due to the granulose swelling up, bursting the cellulose coat, becoming free and dissolving in water = soluble starch or amylin).

It gives a blue colouration with iodine, which disappears on heating and returns on cooling.

It is converted into dextrine and grape-sugar by amylolytic ferments or by boiling with dilute acids.

(B) **Glycogen** :

Preparation.—Apparatus necessary : A solution of potassio-mercuric iodide, made by precipitating a solution of mercuric chloride with potassium iodide, washing the precipitate, and adding it to a boiling solution of potassium iodide till the latter is saturated. Any precipitate which occurs on cooling is to be filtered off. Dilute hydrochloric acid. Methylated spirit, a large bottle ; ether ; absolute alcohol. Large funnel and Swedish filter-papers. Large knife ; capsule ; several beakers ; distilled water ; ice. Mortar and pestle ; large Bunsen's burner.

Glycogen, which is usually obtained from the livers of animals, is also present to a considerable extent in the muscles of very young animals.

To prepare glycogen, it is best to use the liver of a large rabbit. The animal must have been well fed on a diet of grain and sugar for some days, preferably weeks, previously, and should have a full meal of grain, carrots, and sugar, about two hours before it is killed, in order that it may be in full digestion. The rabbit is killed either by decapitation or by a blow on the head, the abdomen is rapidly opened, and the liver is torn out and chopped up as quickly as possible with the knife, and is thrown, with the exception of a piece preserved to show the presence of sugar, into water which is kept boiling. This operation must be performed within half a minute of the death of the animal, and the water must not be allowed to fall below the boiling-point. The liver is to remain in the boiling water

for five minutes ; it is then placed in a mortar, reduced to a pulp, and again boiled in the capsule for ten minutes. The liquid is filtered, and the filtrate is rapidly cooled by placing the vessel in iced water. The albuminous substances in the cold filtrate are precipitated by adding potassio-mercuric iodide and dilute hydrogen chloride alternately as long as any precipitate is produced. The mixture is then stirred, is allowed to stand for five minutes, and is filtered. Alcohol is added to this second filtrate until glycogen is precipitated, which occurs after about 60 per cent. of absolute alcohol has been added. The precipitate is then filtered off and is washed with weak spirit, strong spirit, absolute alcohol (two or three times), and finally with ether. It is then dried on a glass plate at a moderate heat, and, if pure, should remain as a white amorphous powder. If the water has not been completely removed, the glycogen will form a gummy mass ; in this case it must again be treated with absolute alcohol.

Properties.—It is freely soluble in water, the solution looking opalescent and giving a port-wine colouration with iodine, which disappears on heating and returns on cooling.

It is insoluble in absolute alcohol and in ether.

It exists in the liver during life, but very soon after death is changed into sugar. Prove this by boiling the piece of liver which was put aside in the preparation of glycogen and which was not at once thrown into boiling water ; filter and test the filtrate for sugar.

It is converted into sugar by ferments, or by boiling with dilute acids.

(c) **Dextrine :**

Preparation.—Is made in commerce by heating dry potato-starch to a temperature of 400°. It is also produced in the process of the conversion of starch into sugar by diastase, and by the salivary and pancreatic ferments.

Properties.—A yellowish amorphous powder, white when pure, often with peculiar smell.

It is soluble in water.

It is insoluble in absolute alcohol and in ether.

It corresponds almost exactly in tests with glycogen ; but one

variety (achroo-dextrine) does not give the colouration with iodine.

(D) **Glucose** occurs widely diffused in the vegetable kingdom, in diabetic urine, in the blood, etc.; it is usually obtained from grape-juice, honey, or carrots.

Properties.—It is easily soluble in water ; not so sweet as cane-sugar.

It is not easily charred by strong sulphuric acid.

It is soluble in alcohol.

Tests.—With a solution of glucose show the following tests :

(i.) *Trommer's.*—Add an excess of caustic potash and then a solution of copper sulphate, drop by drop, to the solution, in a test-tube, as long as the blue precipitate which forms re-dissolves on shaking the tube. Heat the upper portion of the fluid, and a yellowish-brown precipitate of copper suboxide appears.

(ii.) *Moore's.*—Heat the solution of sugar in a test-tube with caustic potash ; a brown colouration appears.

(iii.) *Fermentation.*—If a solution of sugar be kept in a warm place for some time after the addition of yeast, the sugar is converted into alcohol and carbon dioxide. ($C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$).

(iv.) *Bismuth or Böttcher's test.*—Add a little bismuth oxide or subnitrate and an excess of caustic potash to the solution in a test-tube, and heat ; the solution becomes at first grey and then black.

(v.) *Picric acid test.*—To the solution add about a fourth of its bulk of picric acid (saturated solution) and an equal quantity of caustic potash, and boil ; the liquid becomes of a very deep coffee-brown.

(vi.) *Indigo carmine test.*—To the solution add a strong solution of sodium bicarbonate, and then a little sulphindigotate of carmine. Warm : the blue colour disappears, but returns on shaking the test-tube.

For the *Quantitative estimation of sugar* see p. 189.

(E) **Lactose** is contained in milk (p. 180).

Properties.—It is less soluble in water than glucose.

It has a gritty and slightly sweet taste.

It is insoluble in absolute alcohol.

It undergoes alcoholic fermentation with extreme difficulty.

It gives the tests similar to glucose, but less readily.

(F) **Inosite** is a non-fermentible variety of glucose occurring in the heart and voluntary muscles, as well as in beans and other plants.

It crystallizes in the form of large colourless monoclinic tables, which are soluble in water, but insoluble in alcohol or ether. It may be detected by evaporating the solution containing it nearly to dryness, and by then adding a small drop of a solution of mercuric nitrate, and afterwards evaporating carefully to dryness, a yellowish-white residue is obtained; on further cautiously heating, the yellow changes to a deep rose-colour, which disappears on cooling, but reappears on heating. If the inosite be almost pure, its solution may be evaporated nearly to dryness. After the addition of nitric acid, the residue mixed with a little ammonia and calcium chloride, and again evaporated, yields a rose-red colouration.

CHAPTER III.

OILS AND FATS.

Oils and fats are neutral substances, with the composition of a compound ether, glycerin, $C_3H_5(OH)_3$ being the alcohol :

Are lighter than water, sp. gr. ·91 to 94 ;

Give a greasy stain on paper ;

Are insoluble in water ;

Are soluble in ether, chloroform, turpentine, or alcohol.

Glycerine.—A sweet-tasted viscid liquid, soluble in water and in alcohol ; insoluble in ether.

Test.—Heat a little glycerine in a test-tube with some solid acid potassium sulphate ; *acrolein*, a body of pungent smell, is produced ($C_3H_8O_3 - 2H_2O = C_3H_4O$).

Olive Oil (glycerine oleate) $\left\{ \begin{matrix} 3(C_{18}H_{34}O_2) \\ C_3H_5 \end{matrix} \right\}$

(i.) Shake up a few drops of olive oil in a test-tube with water and then allow it to stand ; note that no emulsion or mixture takes place. Add ether, and shake the test-tube ; note the solution of the oil in that reagent.

(ii.) Add to some oil and water a few drops of caustic potash and warm. Complete solution takes place, a potassium oleate or soap is formed, and glycerine liberated. Both the soap and glycerine are soluble in water.

(iii.) Add some lead oxide to some olive oil and water in a porcelain basin, and apply heat, stirring the mixture. *Lead oleate* is formed and glycerine is liberated (lead oleate is lead soap).

Fat.—(i.) Heat some fat in a test-tube, with water, until melted; add caustic soda. The fat is saponified, stearate of sodium and glycerine being formed.

(ii.) Add sodium chloride to the solution; *sodium stearate* separates out.

(iii.) Add acid to another portion; sodium stearate is decomposed. *Stearic acid* separates out.

(iv.) To another portion add a solution of calcium chloride; *calcium stearate* is precipitated.

CHAPTER IV.

HEALTHY URINE AND ITS CONSTITUENTS.

HEALTHY URINE.

Is a perfectly transparent amber-coloured liquid, with a peculiar and characteristic, but not disagreeable odour, a bitterish taste, and a slightly acid reaction. The specific gravity varies from 1015 to 1020 ; under exceptional circumstances it may be as low as 1001, and as high as 1025. A light cloud of mucus may often be seen in urine after standing.

Test its reaction with litmus-paper.

Take its sp. gr. with the urinometer.

Constituents.—Its characteristic constituent is

(A) **Urea** (CON_2H_4).—Evaporate urine to half its bulk, filter, and add pure strong nitric acid ; impure urea nitrate separates out. To obtain *pure* urea from this, purify it by re-crystallisation with animal charcoal and afterwards decompose it with barium carbonate into barium nitrate and urea. Evaporate the mixture to dryness and extract with hot alcohol. The pure urea will separate out from the alcoholic solution on cooling. [See also Appendix.]

Properties of Urea.—It is soluble in water and in alcohol. Make a strong solution of urea in water, and use it for the following experiments :

(i.) Allow a few drops to crystallise on a glass slide, and examine with the microscope. Note that the urea crystallises out in transparent four-sided prismatic needles, terminated by one or two oblique facets.

(ii.) Either in a test-tube, or on a glass slide, add a few drops of *pure* nitric acid to some of the urea solution. *Urea nitrate* separates out ($CON_2H_4.2HNO_3$) in the form of six-sided tables. Examine the crystals with the microscope.

(iii.) Proceed in a similar manner to ii., but use a concentrated solution of oxalic acid. *Urea oxalate* separates out ($CON_2H_4.C_2H_2O_4$) in the form of tabular plates, or prismatic bundles. Examine the crystals with the microscope.

(iv.) To a concentrated solution of urea in a test-tube add mercuric nitrate solution ; a white precipitate of a compound of mercuric oxide and urea is formed.

(v.) Proceed as in iv., but previously add some common salt. The precipitate does not form until a large quantity of the reagent has been added ; as the sodium chloride causes the mercuric nitrate to split up into mercuric chloride and sodium nitrate and until the whole of the sodium chloride has been used up there is no unchanged mercuric nitrate to produce the precipitate.

(B) **Uric Acid** ($C_5H_4N_4O_3$) is another characteristic constituent of urine, from which it may be obtained thus : Add five or six drops of pure nitric acid to two ounces of urine in a narrow urine glass, and allow it to stand for twenty-four hours. A red brick-coloured sediment of uric acid will be observed. Examine some under the microscope. Brown crystals of various sizes will be seen, the most characteristic being plates, rosettes, and lozenge-shaped.

Properties of Uric Acid.—It is insoluble in cold water, very slightly soluble in hot, soluble in caustic alkaline solutions, forming urates, most easily soluble in solutions of lithium salts.

With uric acid powder show the following tests :

(i.) *Murexide test.*—Add a drop of strong nitric acid, evaporate to dryness over water-bath, or at a temperature not exceeding 40° C. *Alloxan* ($C_4H_2N_2O_4$) is formed, as a reddish-coloured residue. Add a drop of ammonia solution, and the purple colour of *murexide* ($C_8H_8N_6O_6$) is produced.

(ii.) *Schiff's test.*—Dissolve uric acid in a solution of sodium carbonate, and place a drop upon a filter-paper moistened with solution of silver nitrate ; a black stain will result.

(C) **Phosphates** (earthy).—Mix equal quantities of caustic

potash and urine in a test-tube. A transparent flaky precipitate will separate.

(D) **Chlorides.**—Add solution of silver nitrate to urine, a curdy, yellowish precipitate will fall of chloride and phosphate of silver; add nitric acid, the phosphate will dissolve, leaving behind the chloride, which is soluble in ammonia. The phosphates may also be shown by adding molybdate of ammonium and boiling. A yellow colour or precipitate will occur.

(E) **Sulphates.**—To urine acidulated with hydrochloric acid, add barium chloride solution. A precipitate of barium sulphate will occur, insoluble in nitric acid.

(F) **Colouring matter** may be precipitated with the chlorides, sulphates, phosphates on addition of lead acetate.

Quantitative estimation of Urea. — (i.) *Hypobromite method.*—One of the forms of apparatus* employed in this method (Russell and West's) consists of (*a*) a water-bath supported by three iron bands, arranged as a tripod. The bath is provided with a cylindrical depression, and with a hole, into which fits a perforated india-rubber cork; (*b*) a bulb tube with a constricted neck; (*c*) a glass rod provided with an india-rubber band at one extremity; (*d*) a pipette of five cubic centimetres capacity; (*e*) a graduated glass collecting tube; (*f*) a spirit lamp; (*g*) a wash-bottle with distilled water; (*h*) hypobromous solution. The hypobromous solution is made in the following way: three and a half ounces (100 grm.) of solid caustic soda is dissolved in nine ounces (250 grm.) of distilled water. When the solution is cold, seven drachms (25 c.c.) of pure bromine are to be added carefully and gradually. The mixture is not to be filtered: it keeps badly, and for this reason it should be made shortly before it is required; or the solution of caustic soda in water may be made in large quantities as it does not undergo any change, the bromine in the proper proportion being added at the time it is required for use.

Method.—Fill the pipette to the mark on the stem with the urine to be examined; pour the 5 c.c. of urine thus measured out into the bulb; fill up the bulb tube as far as the constricted neck

* Made by Cetti, Brook Street, W.C.

with distilled water from the wash-bottle ; insert the glass rod (*c*) in such a way that the india-rubber band at the extremity fills up the constricted neck ; the diluted urine should exactly occupy the bulb and neck of the tube, no bubble of air being below the elastic band on the one hand, whilst on the other the fluid should not rise above the band ; in the former case a little more water should be added, in the latter a fresh portion of urine must be used, and the experiment repeated. After adjusting the glass rod, fill up the rest of the bulb tube with hypobromous solution ; it will not mix with the urine so long as the rod is in place. The water-bath having been previously erected, and the india-rubber cork fixed firmly into the aperture, the bulb tube is to be thrust from below through the perforation in the cork. The greater part of the tube is then beneath the water-bath, the upper extremity alone being grasped by the cork. Fill the water-bath half full of water, fill also the graduated glass tube (*e*) with water, and invert it in the bath ; in doing this no air must enter the tube, which when inverted should be completely filled with water. Now slide the graduated tube towards the orifice of the bulb tube, at the same time withdrawing the glass rod which projects into the bath through the cork. At the instant that the rod is withdrawn the hypobromous solution mixes with the diluted urine, and a decomposition takes place represented thus :

$$CON_2H_4 + 3NaBrO + 2NaHO = 3NaBr + 3H_2O + Na_2CO_3 + N_2.$$

Urea + sodium hypobromite + caustic soda = sodium bromide + water + sodium carbonate + nitrogen.

The nitrogen produced is given off as gas, and displaces the water in the graduated tube, which is held over it. The gas is at first evolved briskly, but afterwards more slowly ; to facilitate its evolution, the bulb of the tube may be *slightly warmed* with a spirit lamp ; as a rule, however, this is unnecessary. After ten minutes, the amount of water displaced by the gas should be read off on the tube, which is divided into tenths. Each number on the tube represents one gram of urea in 100 c.c. of urine. Normal urine should yield roughly 1·5-2·5 parts of nitrogen by this test. If 5 c.c. of urine gives off more nitrogen than fills the tube to iii., dilute the urine with an equal volume of

water, and take 5 c.c. ; read off and multiply by two. If the urine contain albumen, heat it with two or three drops of acetic acid, filter, and take 5 c.c. of the filtrate.

(ii.) *Liebig's Method.*—This method is of greater accuracy. *The solutions required are* (*a*) *baryta mixture* = 2 vols. of saturated solution of barium nitrate and 1 vol. of saturated solution of barium hydrate ; (*b*) standard solution of *mercuric nitrate*, such that 1 c.c. will precipitate ·01 grm. of urea, and (*c*) a solution of *carbonate of soda.*

Method.—Take 40 c.c. of urine, add 20 c.c. of (*a*), filter off the precipitate of sulphates and phosphates ; keep the filtrate. Fill a burette with (*b*), and take 15 c.c. of the filtrate in a dish. Let (*b*) fall drop by drop into the 15 c.c. in the dish, stirring constantly. Have ready a glass plate with several separate drops of (*c*), and from time to time add a drop of the urine mixture by means of a glass rod to one of the drops. When a yellow colour *first* appears in a drop of the $NaCO_3$, the mercuric nitrate is just in excess. Read the burette. Calculate as follows :

1 c.c. of mercuric solution precipitates ·01 grm. of urea ∴ the No. of c.c. used × ·01 = amount of urea in 15 c.c. of filtrate, *i.e.* in 10 c.c. of urine. But 10 c.c. of urine usually contains enough NaCl to act on 2 c.c. of mercury solution. Hence, *when reckoning the number of c.c. of standard mercury solution used, a deduction of 2 c.c. must always be made.*

Quantitative Estimation of Chlorides :

Liebig's Method.—The solutions required are (*a*) *baryta mixture* as above ; and (*b*) standard solution of *mercuric nitrate*, such that 1 c.c. would be capable of decomposing ·01 grm. of sodium chloride.

Method.—Take 40 c.c. of urine free from albumen, and add 20 c.c. of (*a*). Filter. Take 15 c.c. of filtrate and place in a flask or dish, adding a drop or two of nitric acid. Fill a burette with (*b*), and slowly run some of this solution into the filtrate in the dish, stirring constantly. *As soon as* a distinct cloud appears in the diluted urine, and does not disappear on stirring, then all the sodium chloride in urine has been decomposed. Read burette. Calculate as follows :

1 c.c. of mercury solution decomposed ·01 grm. of NaCl, ∴ the number of c.c. used × ·01 grm. = number of grms. of NaCl in 15 c.c. of filtrate, *i.e.*, 10 c.c. of urine.

Quantitative Estimation of Phosphates :

The solutions required are (*a*) solution of *sodium acetate*, containing 100 grm. of sodium acetate, 100 c.c. of acetic acid, and 900 c.c. of distilled water ; (*b*) a solution of *uranium acetate* or *nitrate*, such that 1 c.c. will precipitate ·005 grm of phosphoric acid ; and (*c*) a solution of *ferrocyanide of potassium.*

Method.—Take 50 c.c. of urine. Add some (*a*) solution, and heat on water-bath to nearly 100° C. Fill burette with (*b*), and allow this to fall into the urine slowly. Have ready a glass plate with several distinct drops of potassium ferro-cyanide solution. From time to time add a drop of urine mixture to one of the drops ; and when there FIRST appears a reddish-brown colour in a drop of potassium ferro-cyanide, all the phosphates are precipitated. Read burette. Calculate thus :

1 c.c. precipitates ·005 grm. of phosphoric acid, ∴ the number of c.c. used × ·005 grm. = number of grms. of phosphoric acid *in* 50 *c.c. of urine.*

CHAPTER V.

(A) THE BLOOD.

(i.) TEST *the alkaline reaction of the blood* in the following way, as recommended by Schäfer :—A drop of blood, obtained by pricking the finger, is placed upon the smooth-coloured surface of a piece of dry, faintly reddened, glazed litmus-paper, and after a few seconds is wiped off with the corner of a handkerchief or clean linen rag moistened with water. The place where the blood has stood is seen to be marked out as a well-defined blue patch upon the red or violet ground.

(ii.) Show that the *coagulation of blood is retarded* by freezing and by the presence of neutral salts. Draw a few drops of blood from the finger into a watch-glass previously cooled in a freezing mixture ; no coagulation takes place at the freezing temperature. Add a drop of blood to a little saturated solution of sulphate of soda in a watch-glass ; the blood does not coagulate.

Two c.c. of blood are placed in a platinum capsule, which is surrounded by alternate layers of pounded ice and salt. The capsule is allowed to remain until the blood is frozen ; it is then removed, and the solid mass of frozen blood is gradually thawed. The blood on again becoming liquid will be found to be darker in colour and more transparent than it was previous to congelation. It is in the *lake* condition, owing to the discharge of the hæmoglobin from the corpuscles into the plasma.

The *tests* for blood are (iii.) the examination by the spectroscope when oxyhæmoglobin and its derivatives give characteristic absorption bands, to be presently mentioned (p. 174).

(iv.) The formation of oxyhæmoglobin crystals (p. 55).

(v.) The formation of hæmin crystals (p. 56).

(vi.) *The guaiac test.*—A drop or two of tincture of guaiacum is added, and mixed by shaking with the suspected fluid ; to the mixture is added some ozonic ether ; if blood be present a sapphire-blue colour appears at the junction of the fluids, and mounts upwards through the ozonic ether, bubbles of gas being at the same time evolved.

Hæmoglobin and its Derivatives.—(i.) Dilute defibrinated blood with ten or twelve times its bulk of distilled water, place some of the solution in a test-tube (12 c.m. × 2 c.m.) or other suitable vessel, and examine with spectroscope. The whole of the spectrum will be seen to be cut off except the red. Dilute some of the above four times, and it will be found that an absorption band extends from D (sodium band) to a little beyond E (middle of green) ; but that the red, orange, yellow and part of the green will be seen. On further dilution (five times and beyond) the broad band will be found to be replaced by two narrower bands separated by a green interspace. Of the two bands, the one to the left next to D is the narrower and deeper. On extreme dilution with water both of the bands disappear, but of the two the band D persists the longer. Before the bands disappear they are seen as faint shadows. These appearances indicate the presence of *oxyhæmoglobin.*

(ii.) To a solution of blood (1 in 20) add a few drops of ammonium sulphide* (NH_4HS) and thoroughly mix ; the solution becomes purplish and on spectroscopic examination the single band of *reduced hæmoglobin* will be seen. It is fainter and with less sharply defined edges than those of oxyhæmoglobin, extends from D to E, none of the green being visible in the midst. On dilution the band soon disappears, but as long as it remains, continues single. There is in addition more absorption to the left and less to the right end than in (i.).

(iii.) Pass CO for some time through blood diluted as in (ii.) On examination the two bands of *carbonic oxide hæmoglobin* may be observed. These bands are almost exactly similar to

* This we find to be the most convenient reducing agent, but Stokes' fluid, a solution of ferrous sulphate, to which is added tartaric acid, and then NH_4HO until reaction is alkaline, or a solution of a stannous salt treated in the same way, may be used.

those of oxyhæmoglobin, but are slightly nearer the violet end, and are besides more equal and deeper. The solution resists reducing agents. Nitric oxide gas has much the same effect.

(iv.) To a further portion of diluted blood add some drops of acetic acid, and boil. The liquid becomes brown and the spectrum shows a distinct band in the middle of the red (to the right of C), indicating the presence of *acid hæmatin*. After cooling, add ether and shake up well. The ether on settling at the top is seen to be of a brown colour, and on examination shows three bands, besides the C band, viz., a very faint narrow band to right of D, a broad band to left of E, also a faint and third about midway between E and F.

(v.) As in (iv.), but add excess of ammonia ; a band is seen in the red, but nearer D (*alkaline hæmatin*).

(vi.) To liquid (v.) add a reducing agent ; a spectrum showing two bands somewhat like those of oxyhæmoglobin, but more to the right, is seen : *Stokes' reduced hæmatin or hæmo-chromagen.*

(vii.) Dissolve some dried blood in saline solution. It will show a spectrum of *methæmoglobin*, consisting of the two bands of oxyhæmoglobin; another band in the red near C, like that of (iv.). On the addition of ammonia the last band is replaced by one close to the left of D. On addition of ammonium sulphide the spectrum of (ii.) will appear, and on shaking with air that of (i.)

(viii.) Dissolve a little fresh blood in strong sulphuric acid, and, if necessary, filter through asbestos ; a beautiful deep red-coloured fluid is the result—*hæmatoporphyrin.* It gives two bands, one at D, and a dark well-defined band about midway between D and E.

Estimation of the Colouring Matter of the Blood.—*Hæmoglobinometer.** A method of approximately estimating the amount of the hæmoglobin in blood has been devised by Dr. Gowers, with an instrument called a *hæmoglobinometer.* The theory of the apparatus consists in comparing a given sample of the blood to be examined, diluted with given quantities of water, with a standard colour solution representing the colour of a normal (1 per cent.) solution of blood. The apparatus con-

* Made by Hawkesley, Oxford Street.

sists of two glass tubes of exactly the same size. One contains (D) a standard of the tint of a dilution of 20 c. mm. of blood, in 2 c.c. of water (1 in 100), composed of glycerine jelly tinted with carmine and picrocarmin. The second tube (C) is graduated, 100 degrees being equal to 2 c. (100 times 20 c. mm.).

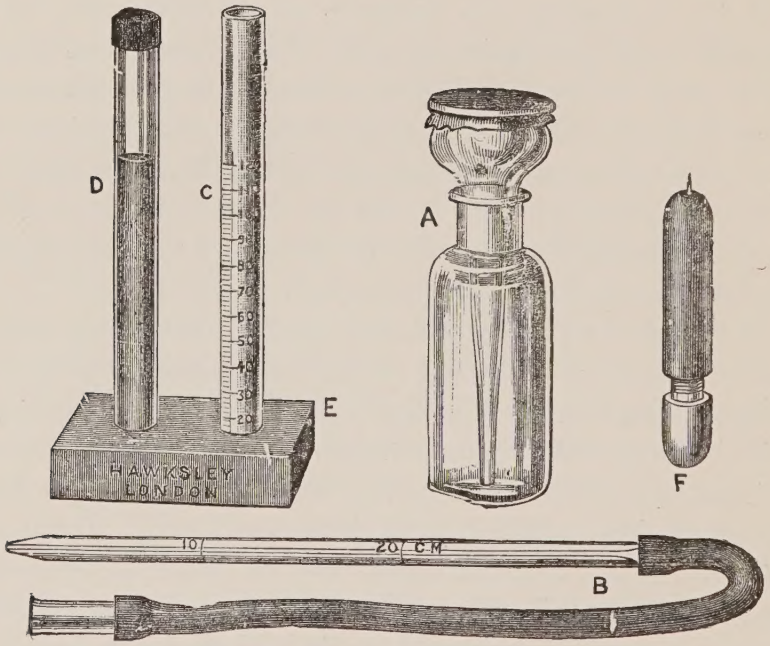

Fig. 25.—The Hæmoglobinometer (Gowers).

The 20 c. mm. of blood are measured by a capillary pipette (B) (similar to, but larger than, that used for the hæmacytometer). This quantity of the blood to be tested is ejected into the bottom of the tube, a few drops of distilled water being first placed in the latter. The mixture is rapidly agitated to prevent the coagulation of the blood. Distilled water is then added drop by drop (from the pipette stopper of a bottle (A) supplied for that purpose), until the tint of the dilution is the same as that of the standard, and the amount of water which has been added (*i.e.* the degrees of dilution) indicates the amount of hæmoglobin.

Since average normal blood yields the tint of the standard at 100 degrees of dilution, the number of degrees of dilution necessary to obtain the same tint with a given specimen of

blood is the percentage proportion of the hæmoglobin contained in it, compared with the normal. By ascertaining with the hæmacytometer the corpuscular richness of the blood, we are able to compare the two. A fraction, of which the numerator is the percentage of hæmoglobin, and the denominator the percentage of corpuscles, gives at once the average value per corpuscle. In using the instrument, the tint may be estimated by placing a piece of white paper behind the tubes ; some light is, however, reflected from the suspended corpuscles from which the hæmoglobin has been dissolved. It will be found that during six or eight degrees of dilution it is difficult to distinguish a difference between the tint of the tubes. It is therefore necessary to note the degree at which the colour of the dilution ceases to be deeper than the standard, and also that at which it is distinctly paler. The degree midway between these two will represent the hæmoglobin percentage.

Serum :

(i.) Take some clear serum in a large test-tube, and add to it an excess of solid magnesium sulphate or sodium chloride. Shake violently. A flocculent precipitate of *paraglobulin* is obtained ; separate it by filtration.

(ii.) Dissolve the precipitate by washing the filter containing it with a small amount of distilled water (it being soluble in dilute saline solution, which is thus made by the water dissolving the adhering salt), and with part of the solution ascertain the temperature at which the globulin coagulates. Retain the remainder (α).

(iii.) In filtrate from (i.) show presence of *serum-albumin* by heat, noting the temperature at which it coagulates. Filter.

(iv.) Test filtrate from (iii.) for any *proteid remainder*, also for *chlorides* and *phosphates*.

(v.) Dilute serum with ten times its volume of water and pass a brisk stream of CO_2 through the solution ; a precipitate of paraglobulin will appear. Separate by filtration, and dissolve as in (ii.). To one portion of the solution apply the globulin tests ; retain the second portion (β).

Hydrocele Fluid :

(vi.) Take some hydrocele fluid and treat as in (i.) ; a precipitate of *fibrinogen* occurs. Separate it.

(vii.) Dissolve *fibrinogen*, as in (ii.), and proceed in exactly similar manner, and taking one portion of solution, find the temperature at which it coagulates, and retain the other portion (γ).

(viii.) As in (iii.), show the presence of serum-albumin, and after its precipitation and removal by heat and filtration, test the filtrate for any proteid residuum, and for chlorides.

(ix.) Precipitate fibrinogen by the CO_2 method as in (v.) ; apply the globulin tests to one portion and retain the other (δ).

Coagulation :

(x.) Take fresh serum and add it to an equal quantity of fresh, clear hydrocele fluid (faint yellowish to green in colour, S.G. 1016-1022), and keep it at a temperature of about 37° C. After a variable time a clotting will occur.

(xi.) Add together solutions (α) and (γ) ; clotting will most likely occur (if the fluids from which they have been obtained were fresh).

(xii.) Add together solutions (β) and (δ) ; clotting does not occur.

(xiii.) Allow horse's blood to flow into the saturated solution of magnesium sulphate or sodium chloride and thoroughly mix ; set aside in ice for a day or two. Draw off supernatant liquor of so-called salted plasma, dilute one portion with many times its bulk of water, or place in dialyser ; a clot will after a while form if kept at about 37° C. Saturate the other with solid magnesium sulphate or sodium chloride ; a precipitate of *plasmine* will form.

(xiv.) Treat plasmine as in (ii.), and the solution should clot.

(xv.) Treat plasma with an equal volume of 13 per cent. solution of sodium chloride ; fibrinogen will be separated ; remove by filtration and dissolve as above, and then add solid sodium chloride to saturation, and paraglobulin falls. Remove

by filtration and dissolve. The solutions when mixed should after a time clot.*

Fibrin : See p. 155.

(B) MILK.

An opaque white fluid :

(i.) Examine a drop of milk under the microscope with a high power. See that it consists of fat globules of different sizes in a clear fluid. Add dilute acetic acid by irrigation, and observe the coalescence of the globules owing to the excess of acid dissolving their casein membrane.

(ii.) Test the alkaline reaction of *fresh* milk (London milk may be slightly acid) ; it becomes acid on standing, owing to the formation of lactic acid by fermentation, whilst in consequence the casein separates.

(iii.) Take the specific gravity of fresh milk with the hydrometer, 1025-30—skimmed milk is higher. To 50 cc. of skimmed milk add water gradually from a burette till the specific gravity is brought down to normal. Read off the amount of water required.

The *constituents* whose presence is to be demonstrated are *oil* or *fat*, *casein*, *serum-albumin*, *lactose* or *milk sugar*, and *salts*.

(iv.) *Fat.*—To a portion of milk, add its own volume of caustic potash or soda, and warm the solution *gently ;* the oil globules will be set free from their enveloping casein. Cool, add ether, shake the mixture, and allow it to stand. The fat will be dissolved in the ether, and will form with it a clear superstratum. Remove the transparent top layer with a pipette, evaporate off the ether ; the oil will be left, and will give the *characteristic greasy spot when dropped upon paper.*

(v.) *Casein.*—Dilute some of the milk with its own bulk of water ; add a few drops of dilute acetic acid until a slight granular precipitate is formed. Warm the solution *gently* to 40° C., and a copious flocculent precipitate will appear. Filter off, and label the precipitate 'A.'

(vi.) a. *Serum albumin.*—Boil the clear filtrate (from A); a

* Many other experiments on coagulation may be done if time permit, *e.g.*, use of fibrin ferment.

coagulum of albumin will be formed. Filter this off, and label the precipitate 'B.'

b. *Acid-albumin.—Exactly* neutralise the clear filtrate (from B), with caustic potash or soda ; a precipitate of albumin which is soluble in acids may appear.* Filter off precipitate, and label it 'C.'

(vii.) *Lactose.*—Test the clear filtrate (from C) by Trommer's test for sugar.

(viii.) To precipitate A in a test-tube add nitric acid, the precipitate is dissolved ; boil, and when cold add strong ammonia ; the solution becomes orange coloured (*xanthoproteic test*).

(ix.) To a second portion of precipitate A add sodium phosphate ; the precipitate will be dissolved : add dilute acetic acid to the solution till a neutral reaction is just obtained ; no precipitate occurs : add more acetic acid ; a precipitate is thrown down (since acid albumin is now present)—*casein or alkali-albumin is present.*

(x.) To a third portion dissolved in caustic soda or potash add lead acetate ; a black precipitate of lead sulphide is formed. Therefore sulphur is present ; consequently *casein*, and not artificially produced alkali-albumin, is present.

(xi.) Test the precipitate on filters B and C, by boiling with *Millon's reagent;* a pink colouration will in each case be produced, showing that albumen is present.

(xii.) Test a second portion of the clear filtrate from C for (i.) *phosphates*, by the addition of ammonio-sulphate of magnesia; a precipitate is formed : also by molybdate test ; and for (ii.) *chlorides* by the addition of silver nitrate to another portion which has been acidulated with nitric acid ; a curdy precipitate will fall, soluble in ammonia.

(xiii.) Milk may be curdled in several ways :

(*a*) By *rennet.*—Add some extract of rennet to some milk warmed to about 45° C. Observe : the *coagulum* of casein.

(*b*) By *acid*, as above. — Take two portions of curd precipitated by acid ; dissolve one in caustic soda

* This acid albumin has been produced by the previous acidification of the milk in the precipitation of casein.

and the other in lime-water, and filter each. Add some rennet to both filtrates, and observe the result.

Lime solution coagulates.

Soda „ does *not* coagulate.

(*c*) By *salt*.—Shake some milk in a test-tube with solid sodium chloride or magnesium sulphate ; filter and dissolve (Qy.) some of the curd in water and add rennet. Observe that it *coagulates*.

(C) THE BILE.

The bile is a more or less viscid fluid of a colour varying from deep yellow to green or brown.

(i.) Test its reaction. Alkaline or neutral ; and its S.G.—1018-20.

(ii.) Neutralise and boil. There is no precipitate, which indicates the absence of *albumins*.

(iii.) Dilute with water and acidify with acetic acid. There will be a precipitate of mucin. Filter. (Dissolve the precipitate on the filter in lime-water, and test with *Millon's reagent* and with *lead acetate*).

(iv.) To a portion of the filtrate from (iii.) apply Gmelin's test for *bile pigments*.

Gmelin's Test for Bile Pigments.—Place a drop of bile on a white plate, and add a drop of strong yellow nitric acid to it. A play of colours is produced. Green, blue, violet, red, and yellow appear in succession.

(v.) To another portion of the filtrate apply Pettenkofer's test for *bile salts*.

Pettenkofer's Test for Bile Salts.—Add a few grains of white sugar, or a drop or two of syrup, to a solution of bile in a test-tube, shake well, add strong sulphuric acid, and cool. A reddish-purple colour is produced.

(vi.) To another portion of the filtrate add chloroform and warm slightly. This extracts the colouring matter (*bilirubin*). Remove the chloroform solution with a pipette and examine it with spectroscope. Observe : *absence of absorption bands* Apply Gmelin's test.

(vii.) Bile shaken up with oil divides it into very small globules (an emulsion being formed). This can under very slight pressure pass through animal membranes; oil alone cannot.

(viii.) Take two filters, wet the one with water, the other with bile, and pour a small and equal amount of oil on each. Oil passes slowly through the one wetted with bile, not at all through the other.

(ix.) Bile precipitates pepsin and peptones. Add a considerable quantity of bile to a solution of fibrin digested in artificial gastric juice; a precipitate of pepsin and peptone will fall, and the solution will be found to have lost its proteolytic properties.

Bile Acids, Glycocholic ($C_{26}H_{43}NO_6$), and Taurocholic ($C_{26}H_{45}NSO_7$).

Preparation.—Mix bile, which has been evaporated to one-fourth of its bulk, with animal charcoal, evaporate to perfect dryness on a water bath, and extract it, whilst still warm, with absolute alcohol. The alcoholic filtrate should be colourless; if this is not the case, more charcoal must be added. The alcohol is distilled off, and the dry residue is treated with absolute alcohol. The alcohol is then filtered off, and to the filtrate anhydrous ether is added as long as a precipitate is thrown down. The solution and precipitate are to be set aside in a closely-stoppered bottle for some days, when crystals of *bilin* will be produced. If the reagents were not perfectly anhydrous, a gelatinous mass will be formed, but no crystals. Bilin consists of glycocholic and taurocholic acids, which may be separated from one another by dissolving it in water, and adding first solution of neutral lead acetate, and then a little basic lead acetate. This combines with glycocholic acid to form an insoluble lead glycocholate. Filter, and add to the filtrate basic lead acetate and ammonia; a precipitate of lead taurocholate will be formed, which may be filtered off. In either case the lead must be got rid of by suspending or dissolving in hot alcohol, adding hydrogen sulphate, filtering, and adding water.

Cholesterin.—It is contained in bile, gall-stones, nervous matter, etc.

Preparation.—Usually by extracting powdered gall-stones with ether or boiling alcohol. Cholesterin crystallizes out on

evaporation of the extract. The insoluble residue consists of bile, colouring matter, and mucin.

Properties and Tests.—Crystallizes in rhombic plates. Examine under the microscope.

It is insoluble in water and cold alcohol ; freely soluble in boiling alcohol and in ether.

It gives a red colour with strong sulphuric acid and with nitric acid and ammonia.

It gives a greenish blue to violet with sulphuric acid and iodine or zinc chloride, and with sulphuric acid and chloroform a play of colours, beginning with blood-red, and ending with green, afterwards disappearing.

Analysis of Gall Stones :

(i.) Make an ethereal extract. Allow a few drops to evaporate on a slide, and examine for cholesterin crystals with a microscope.

To a portion of the extract add strong sulphuric acid ; a bright red colour indicates *cholesterin.*

(ii.) Boil the residue (coloured) in water with a few drops of acid ; pour off the water, and add some warm chloroform.

Examine the chloroform solution for *bilirubin* as above.

CHAPTER VI.

THE DIGESTIVE FLUIDS.

(I.) SALIVA.

PROCURE a copious flow of saliva by touching the tongue with a crystal of tartaric acid, avoiding, as much as possible, any mixture of the acid with the saliva.

Salivary Digestion.—Take five test-tubes containing equal quantities of starch paste, and add to each equal quantities of saliva. (i.) Boil briskly and place in the incubator ; (ii.) Place in ice and salt ; (iii.) Keep at the temperature of the room ; (iv.) Place in the incubator ; (v.) Render distinctly acid and place in the incubator. At the end of ten minutes test all for sugar by Trommer's test. (i.), (ii.), and (v.) will contain no sugar ; (iii.) a little ; (iv.) abundance.

Composition of Saliva :

(A) INORGANIC CONSTITUENTS. — Test qualitatively, small portions, for the following :

1. *Carbonates.*—Shake with a small quantity of any strong acid. Bubbles of CO_2 will be evolved.

2. *Chlorides.*—Acidulate with nitric acid, a precipitate with silver nitrate, soluble in ammonia.

3. *Phosphates.*—A yellow precipitate with ammonium molybdate on boiling.

4. *Sulphates.*—A precipitate with barium chloride or nitrate, which does not disappear on boiling with nitric acid.

5. *Potassium.*—A violet colour in Bunsen flame, visible through blue glass.

6. *Sodium.*—Yellow flame.

7. *Calcium.*—Precipitate by ammonium oxalate.

8. *Magnesium.*—Add ammonium chloride and ammonia first, then sodic phosphate ; a precipitate of ammonio-magnesium phosphate will result.

9. *Potassium Sulphocyanate.*—It gives a reddish colour on adding ferric chloride, which is not altered by hydrochloric acid, but discharged by mercuric chloride (meconic and acetic acids give somewhat similar colour, which is discharged by hydrochloric acid in the former and by mercuric chloride in the latter case).

(B) ORGANIC CONSTITUENTS :

1. Acidulate with acetic acid ; a precipitate of *mucin.* Filter.

2. Apply to some of the filtrate the proteid reactions.

3. Saturate more of the filtrate with solid sodium chloride ; a precipitate of *globulin* appears.

(II.) GASTRIC JUICE.

(A) **Pepsin.**—Show the digestive action of pepsin by means of glycerine extract of pig's stomach or solid pepsin.

Take three test-tubes containing (i.) hydrochloric acid solution ·2 per cent. and fibrin ; (ii.) pepsin, water, and fibrin ; (iii.) pepsin, hydrochloric acid ·2 per cent. solution, and boiled fibrin; also (iv.) a flask containing pepsin, hydrochloric acid ·2 per cent., and fibrin, all in larger quantities than above. Place all in incubator for fifteen minutes. Note that in (i.) the fibrin swells and becomes transparent, but does not dissolve ; in (ii.) and (iii.) the fibrin is unchanged; in (iv.) the fibrin is dissolved. Filter (iv.).

Neutralise filtrate ; a fairly copious precipitate of *syntonin*, *parapeptone* or *anti-albumose* (Kühne) results. Filter. To filtrate add strong nitric acid precipitate of so-called *A peptone* (Meissner) or *hemialbumose* (Kühne). Filter again and add potassium ferrocyanide, a precipitate of B peptone. Filter this, and apply to the filtrate picric acid and test for *C* or *true peptone*, etc. (p. 156).

Reactions of pure Pepsin :

1. Should not be precipitated by nitric acid, tannic acid, iodine, or mercuric chloride.

2. Is precipitated by platinum chloride, and neutral lead acetate.

(B) **Rennet.**—The action of the curdling ferment of the stomach upon milk has already been shown.

(III.) PANCREATIC JUICE.

The digestive action of the pancreas may be tested by means of extracts of the gland of various kinds in many ways. Take the pancreas of a pig, dog, or ox, and remove all adhering fat, chop it up into small pieces and treat in the following ways: (i.) Pound in mortar with distilled or slightly acidulated water, keep at 25° C. for an hour—strain through coarse muslin; (ii.) pound in mortar with powdered glass, slightly acidulate with dilute acetic acid, leave for half an hour, and then add ten to twelve times its volume of glycerine; thoroughly mix. (iii.) Proceed as in (i.), but use brine instead of water; (iv.) make an extract of the fresh gland with water or glycerine; but without acid or long exposure to the air.

[*Instead of these extracts certain commercial preparations may be employed*, e.g., *Benger's Liq. Pancreaticus.*]

1. Add a few drops of one of the above preparations and some sodium carbonate—1 per cent. solution—to some starch mucilage in a test-tube, expose in a water bath at 40° C. for fifteen or twenty minutes, test for sugar. The intermediate products (*erythro-dextrin*, giving a red colour with iodine; and then *achroo-dextrin*, giving no colour with iodine, but precipitated by alcohol) may be tested for, if the action is allowed to go on only for a less time and be stopped by boiling. Experiments proving the conditions under which the ferment acts are similar to those described for the salivary ferment. Do them.

2. In a similar manner place some of the extracts (i.) or (ii.) and (iv.) in test-tubes with the alkaline solution as in 1, and add to each a fragment of boiled fibrin; place them in a water bath as above for half an hour, neutralise, filter, boil and refilter, and test filtrate for peptone; the test-tube containing (i.) or (ii.) will contain more peptone—indicating that extract (iv.) contains less of active ferment.

3. As in 2, but boil one solution; no solution of fibrin takes place; allow the unboiled digestive fluid to remain in water-bath

for an hour, and watch, from time to time, the gradual erosion of the edges of the fibrin, noting that the fibrin does not swell up and become transparent as in gastric digestion.

4. For intermediate products of pancreatic proteolytic digestion, viz., *hemi and anti-albumose* (*Kühne*) *or alkali-albumin etc.*, neutralise the fluid after half an hour's digestion with weak acid, and a precipitate occurs ; and take out the undissolved fibrin, and show that it is easily soluble in 1 per cent. hydrochloric acid and in 10 per cent. saline solution. The solution in the latter is precipitated by heat and by nitric acid. This shows that the fibrin has been converted into a soluble form, of a globulin type.

5. Add some of the most active extract to a larger quantity of fibrin and sodium carbonate solution in a beaker ; allow the action to go on for two hours at 40° C., then neutralise with acetic acid, filter, boil, and refilter. Evaporate the filtrate to one-third of its bulk, add absolute alcohol while still hot—leave it for twenty-four hours to precipitate peptones—filter, concentrate the filtrate ; *tyrosine* will crystallise out : pour off mother liquid, and concentrate still further ; *leucin* will crystallise out.

6. In a pancreatic digestion fluid, when the action has gone on for twenty-four hours at least, notice its smell, and test for *indol* —by acidulating with dilute sulphuric acid – boiling, and adding a drop of very dilute nitrous acid ; a reddish-pink colour should appear at once or on standing. If the solution give a purple colour with chlorine water, the associated body, *naphthalamine*, is present.

7. Add some of (i.) neutralised if necessary, to melted lard at 37° C., in proportion of 2 to 1 ; rub together over a water-bath—a thick creamy emulsion will result.

(N.B.—Saponification and emulsion of oil by pancreatic extract are neither certain nor satisfactory.)

8. Add some of (iii.) or (ii.) to milk in a test-tube, at temperature of 40° C. ; notice that the casein is soon precipitated, and then dissolved, forming peptone ; also in another test-tube do the same experiment with milk diluted $\frac{1}{4}$; note that precipitation does not occur, but that in a few minutes after the addition of the extract the casein can be precipitated on boiling (*metacasein*) as well as on addition of dilute acid.

(IV.) **THE BILE.** See Chapter V., pp. 181-183.

CHAPTER VII.

UNHEALTHY URINE AND CALCULI.

Albumen present.—(i.) If the urine be neutral or acid, the albumen is precipitated on boiling. If alkaline, render slightly acid with nitric acid or acetic acid, and boil. Albumen is precipitated.

Care must be taken, in testing for albumen in urine, that the test-tube which is employed be clean and free from acid. Perform the following experiment to show the necessity of this precaution. To a small quantity of albuminous urine add an excess of strong nitric acid ; a precipitate of albumen is thrown down. Pour the contents of the test-tube away, and *without* washing it, fill it up with a fresh sample of the albuminous urine. After allowing it to stand five minutes, boil the solution, when, although albumen is known to be present, no coagulum will be formed, since the acid remaining in the test-tube from the previous experiment has been sufficient to convert the albumen into acid albumen. The experiment will frequently, however, be unsuccessful, unless a large excess of the strong acid has been first added.

(ii.) *A more delicate test.*—Acidify urine with acetic acid ; mucus will be precipitated if present. Filter, and add ferrocyanide of potassium to clear filtrate ; a precipitate will be formed in presence of albumen.

(iii.) *Heller's test* (p. 150).

Bile pigment or acid present.—Adopt tests (p. 181).

Uric acid present.—Employ the murexide test (p. 168).

Urates present.—The deposit dissolves on heating; sometimes reappears on cooling. Urates dissolve in caustic alkalies; uric acid is separated on adding strong acids. Apply murexide test to the deposit.

Phosphates present.—The phosphates may be in solution, or may form a deposit. If in solution the urine is feebly acid or neutral. On boiling urine, the phosphates are deposited, the deposit being soluble in weak acid. Deposits of phosphates are insoluble in caustic alkalies.

The phosphates are either in the form of amorphous phosphate of lime ($Ca_3(PO_4)_2$), crystallized phosphate of lime ($2CaH.PO_4$), or ammonio-magnesium phosphate or triple phosphate ($Mg(NH_4)PO_4 + 6H_2O$), sometimes precipitated all together, or the first and third varieties.

The reason why phosphates are deposited on boiling in urine which was before clear is uncertain, but possibly it may be due to the action of heat expelling carbon dioxide, or decomposing urea into ammonium carbonate, which renders the urine alkaline.

Oxalates present.—The deposit is soluble in hydrochloric acid, but insoluble in acetic acid. Examine their crystalline form with a microscope.

Sugar present (Diabetes).—Specific gravity generally high, 1030 to 1050. Apply Trommer's test, or any of the others mentioned (p. 163).

Quantitative estimation of grape sugar.*

1. *Fehling's Method.—Solution required*=copper sulphate and caustic soda, with some sodic potassic tartrate of such a strength that 10 cc. of solution contain the amount of cupric oxide which ·05 grm. of sugar can reduce to cuprous oxide. (This solution should be freshly prepared.)

Method.—Remove any albumen which may be present in the urine by boiling and filtering. Take 10 cc. of the urine, free from albumen, and add 90 cc. of distilled water. Place this in a burette. Put into a flask or dish 10 cc. of the standard solution, and dilute with four times its bulk of water and boil. Run into it, from burette, some of the diluted urine, say 20 cc., and boil. Allow precipitate to settle, and if supernatant fluid is still blue,

* *Fehling's solution* is made as follows: Take of sulphate of copper, 40 grms.; neutral tartrate of potash, 160 grms.; caustic soda (sp. gr. 1·12), 750 grms.; add distilled water to 1154·5 cc. Each 10 c.c. contains ·05 grm. of sugar.

add, say, 5 cc. from burette, and boil again, and so on, till the fluid ceases to have a blue tinge, taking care, towards the end of the process, to add only a few drops each time. If, after adding 20 cc. of diluted urine and boiling, the fluid has been decolourized, too much urine has been added, and another 10 cc. of standard solution must be measured out, running in less than 20 cc. (say 10 cc.) in first instance.

When you have thus determined the number of cc. of diluted urine required to decolourize the solution, that volume contains the amount of sugar necessary to reduce 10 cc. of standard solution, *i.e.*, ·05 grm. But one-tenth only of this is urine, ∴ one-tenth of number of cc. used contains ·05 grm. of sugar. From this you can easily calculate the percentage.

2. *Pavy's Modification of Fehling's Method.*—By Fehling's method it is difficult and tedious to judge of the point of complete reduction of the cupric oxide. Dr. Pavy, accordingly, uses a strongly ammoniacal solution of the above. A certain amount is introduced into a small flask, which is then heated till the vapour of ammonia escapes by a narrow tube. The sugar solution is then allowed to flow from a burette into the flask until the blueness has disappeared, the solution being kept boiling all the time. The blueness is apt to disappear suddenly, and care should therefore be taken towards the end of the process.

Calculate as in Fehling's method.

3. *Estimation of sugar by fermentation.*—Take specific gravity of urine before and after fermentation. Each degree of specific gravity lost by the urine represents one grain of sugar per ounce of urine.

4. Sugar may also be estimated by adding yeast to urine, and collecting the carbon dioxide evolved. The carbon dioxide is a measure of the amount of sugar present.

Blood present.—Examine the deposit formed on standing, with the microscope, for blood corpuscles ; add to another portion a drop of tincture of guaiacum, and about a drachm of ozonic ether ; a blue colour will appear at the junction of the fluids.

Pus present.—Examine the deposit with the microscope. Add caustic potash ; the urine becomes stringy.

Carbolic Acid in Urine.—The urine is dark olive-green or black when first passed ; on standing a deposit resembling altered blood often takes place, and the urine becomes lighter in colour. On the addition of strong sulphuric acid, the odour of tar is exhaled from the urine. The addition of perchloride of iron develops a blue colouration.

Salicylic acid in urine gives a purple colour with the perchloride of iron.

Chylous Urine.—The urine may be clear or milky when passed ; on standing it coagulates, forming a tremulous mass which after a time liquefies. Examine for albumen, molecular fat, and the nematoid worm, *filaria sanguinis hominis.*

URINARY CALCULI AND DEPOSITS.

(i.) Ignite a small portion on platinum foil. If it burn away completely, it is probably uric acid. To confirm this apply murexide test (p. 168).

(ii.) Boil the powdered calculus with distilled water, or, if a urinary deposit, with the supernatant urine.

The powder or deposit may be *dissolved* wholly or partially—or *undissolved.*

(A) **Dissolved** portion consists of

Urates—which are mostly deposited on cooling.

Test for urate of ammonium by boiling with potash to demonstrate presence of ammonia ; and by murexide test for the uric acid. If it is not ammonium urate it is probably potassium or sodium urate ; for either base, test in the ordinary manner.

(B) **Undissolved** portion may consist of

Phosphates,
Calcium oxalate,
Uric acid.

(iii.) Take some of (B), add a few drops of hydrochloric acid, and boil.

(C) **Dissolved.**	(D) **Undissolved.**
Phosphates,	*Uric acid.*
Calcium oxalate.	Confirm by murexide test.

(iv.) Take some of (C) solution and add excess of ammonia; a precipitate will fall in either case: add acetic acid in excess; the precipitate is

(E) **Dissolved.**	(F) **Undissolved.**
Phosphates.	Oxalates.
Confirm by molybdate test.	

If the precipitate in (C) solution is partially dissolved and partially undissolved, phosphates and oxalates are probably mixed.

TABLE OF ABNORMAL URINES.

Urines may be **abnormal** in:

(i.) **Colour**:

Blood (red, or smoky), *carbolic acid* (black), *Bile* (brown), *cystin* (yellowish green) — Too dark.

Albuminous urine., *Chylous* ,, — Too light.

Excess of nitrogenous constituents (orange).

Effects of drugs, e.g., *rhubarb*, red.

(ii.) **Smell**:

Sweet in diabetic urine.

Very rank in urine containing excess of urea or urates.

Ammoniacal in decomposing urine.

When *cystin* is present, *sweetbriar*.

(iii.) **Reaction**:

Alkaline, with excess of phosphates.

Strongly acid, with urates in excess.

(iv.) **Specific Gravity**:

Too high in diabetes mellitus, and in excess of urea.

Too low, in chronic Bright's disease, hysteria, and anæmia.

Abnormal urines may

(A) **Contain no sediment.**	(B) **Contain sediment on standing.**	
Albumen.	Urates.	Cystin.
Phosphates.	Phosphates (sometimes).	Chylous urine (becomes transparent when shaken up with ether; test for albumen).
Sugar.	Pus.	
Bile.	Mucus.	
Carbolic acid.	Oxalates.	
Blood.	Uric acid.	
Salicylic acid.	Albumen (sometimes).	
	Carbolic acid (sometimes).	

(A) **If containing no sediment.**

Boil.

Precipitated.	Not precipitated.
Albumen.	*Sugar.*
Phosphates.	*Bile.*
Blood.	*Carbolic acid.*
	Salicylic acid.

Add nitric acid.

Precipitate dissolved.	Undissolved
Phosphates.	*Albumen.*
	Blood.

With fresh sample of urine apply guaiacum test (p. 174).

Blue colour.	No colouration.
Blood.	*Albumen.*

(Examine with the microscope.)

Apply Gmelin's test (p. 181).

Play of colours.	No play of colours.
Bile.	*Sugar.*
	Carbolic acid.
	Salicylic acid.

Apply Trommer's test (p. 163).

Reduction of copper.	No reduction.
Sugar.	*Carbolic acid.**
	*Salicylic acid.**

* *Apply special tests* (p. 191).

(B) **If containing a Sediment :**

Boil.

Precipitate or sediment dissolved.	Sediment undissolved.
	Phosphates—increased.
	Albumen—increased.
Urates.	*Pus.* *Cystin.*
	Mucus.
	Oxalates.
	Uric acid.
	Carbolic acid (see above).

Add nitric acid.

Precipitate dissolved.	Precipitate undissolved.
Phosphates (soluble in acetic acid).	*Albumen*—increased.
Oxalates (insoluble in acetic acid. Examine sediment with microscope, octahedral crystals or dumb-bells).	*Pus.*
	Mucus—not increased.
Cystin (do. hexagonal plates).	*Uric acid.*

Add caustic potash to fresh portion.

Dissolved.	Undissolved.
Uric acid.	*Albumen.*
Pus (converted into a glairy mass).	
Mucus.	
Apply confirmatory tests.	

CHAPTER VIII.

EXAMINATION OF ORGANIC SUBSTANCES.

THE following notes may be useful. They are not by any means exhaustive of the subject; and the advanced student is advised to supplement them by careful manipulation.

(I.) PROTEIDS.

Plan of examination of a solution containing one or more proteids, with and without other substances.

1. Notice whether clear; if not, filter.

2. To the filtrate or to the original solution apply the proteid reactions, with HNO_3, with Millon's reagent and with copper sulphate and caustic potash.

3. Try the reaction of the filtrate or original solution:

May be (*a*) acid,
(*b*) alkaline,
(*c*) neutral.

If (*a*) or (*b*) carefully neutralize:

A precipitate indicates *acid* or *alkali-albumin*, as the case may be, if, when re-dissolved in dilute acid or alkali, after filtration, the solution gives the proteid reactions.

4. Boil some of the original solution. A precipitate indicates (*d*) a *native-albumin*, or (*e*) a *globulin*.

To distinguish (*d*) and (*e*):

5. Saturate a portion of original solution with solid NaCl or $MgSO_4$.

Precipitated globulin	*Non-precipitated native-albumin*
(Pour some of original solution into distilled water; a distinct precipitate shows [derived-albumins having been excluded] that *myosin* is present).	(Apply special tests for *serum* and *egg-albumin*).

If the presence of native-albumin as well as globulin be suspected, filter, and boil filtrate—coagulation proves it, unless it be slight, when it may be due to imperfect precipitation and separation of globulin.

6. To filtrate from 3, if peptone is suspected as well as acid- or alkali-albumin, or to filtrate from 4 if it is suspected as well as native-albumin or globulin, carefully apply the biuret reaction and other tests (p. 156) for peptones; but remember that gelatin gives almost exactly similar tests, and so put aside some of the solution to cool, and if gelatin be present in sufficient amount, it will solidify; then proceed as in III. 2.

A solution which has been proved to contain one or more proteids may yet contain other organic substances, and to detect these, it is necessary to remove the proteids as far as possible by the above methods and to apply special tests according to II.

If in filtration of the original fluid in 1, a considerable amount remains on the filter, proceed as in IV.

(II.) CARBOHYDRATES.

A solution from which the whole of the proteids, except peptone, has been removed, by boiling with sodium sulphate and acetic acid and filtering, or which is known to contain no proteids, may be examined for carbohydrates as follows:

1. Add *iodine solution.*

Blue colouration, which disappears on heating, and reappears on cooling=*starch.*

Port-wine colouration=*glycogen* or *dextrin.*

2. If peptones be present, remove them by the following method: Evaporate solution to dryness carefully, and add boiling absolute alcohol. Separate the solution from the residue by decantation and filtration. Evaporate off the spirit. Make a watery solution and test for *sugar*.

3. If no peptones be present, apply Trommer's test for *sugar*.

4. If starch or sugar be present in the solution, and glycogen or dextrin is suspected as well, it becomes necessary to add, to a small portion in a test-tube, rather more than its bulk of absolute alcohol. A precipitate of glycogen, which may be redissolved in water and tested with iodine, will occur if it be present. Concentrate the filtrate and add an excess of absolute alcohol. If dextrin be present it will be precipitated.

Other organic substances may still be present. Proceed as in succeeding papers.

(III.) GELATIN, MUCIN, UREA, URIC ACID.

1. Proceed as in I. If the solution give the xanthoproteic and Millon's tests, but none of the other proteid tests, the solution may contain *mucin* or *chondrin* (*tyrosin*).

Add some dilute acetic acid to a portion of solution:

Precipitate = *mucin* or *chondrin*.

Confirm by obtaining a precipitate with alcohol,

No precipitate with tannic acid,

No precipitate with $HgCl_2$.

Mucin and *chondrin* cannot be distinguished from each other.

2. If the solution give the above proteid tests, and also the biuret (purple) reaction, but there be no reaction with ferrocyanide of potassium and acetic acid, and also no precipitate with acetic acid alone, test for *gelatin* (p. 158), and allow the solution to cool; it will gelatinize.

(If tyrosin be suspected, extract with a few drops of hot dilute ammonia and evaporate a portion to dryness; allow it to crystallize, and examine with microscope.)

3. If no reaction has resulted from 1 and 2 and from above, the solution may contain *urea* or *uric acid* (in the form of urates).

To some of solution add NaCl and then $Hg(NO_3)_2$, drop by drop. If no precipitate forms at first, but afterwards appears, *urea* is probably present.

Confirm by obtaining crystals of *urea* and of *urea nitrate*, and examining with microscope.

4. Evaporate some of the solution and apply the *murexide test* for *uric acid*.

(If uric acid be suspected it is better to acidulate some of the solution with HCl, and allow it to stand for twenty-four hours. Uric acid, if present, falls as a crystalline deposit, and may be examined microscopically, or by the murexide test.)

(IV.) EXAMINATION OF A SOLID SUBSTANCE.

1. *Preliminary Examination.*—Observe whether it be amorphous or crystalline (if the latter, examine the crystalline form under the microscope), and apply the xanthoproteic, biuret, and ferrocyanide of potassium and acetic acid reactions. Observe its odour, if any. Thus gain a clue as to the nature of the substance. Then proceed as follows :

If a Proteid or Gelatin :

2. Test solubility in water.

SOLUBLE.	INSOLUBLE.
Native-albumins.	*Other proteids.*
Peptones.	
Gelatin.	

If soluble, apply special tests to the solution ; if not, try solubility in acid, alkali and salt solutions, and apply special tests.

IF NOT A PROTEID:

3. Test its solubility in water (COLD).

SOLUBLE.	INSOLUBLE.
Grape-sugar.	*Starch* (soluble in HOT water).
Milk-sugar.	*Tyrosin* (soluble in HOT water).
Inosit.	*Uric acid.*
Glycogen.	*Cholesterin.*
Dextrin.	(*Fats.*)
Urea.	
Leucin.	

4. If not a proteid, and soluble in water, add iodine solution to a portion of watery solution.

Port-wine colouration = *glycogen* or *dextrin.*

Distinguish glycogen and dextrin by the odour and by their respective solubility in alcohol.

5. If no result with 4, apply Trommer's test to a portion of watery solution. If cupric oxide is reduced = *grape-sugar.*
milk-sugar.

Distinguish by adding absolute alcohol to a portion of the solution (after concentration).

Milk-sugar, white precipitate.
Grape-sugar, no precipitate.

6. If no result with 4 or 5, apply special tests for *urea.*

7. If no result with 6, apply the special test for *leucin* as follows:

Heat in a dry test-tube in Bunsen's flame; a smell of amylamine = *leucin*

(8. If no result, apply special test for *inosit* as follows:
Add HNO_3 to original substance, evaporate carefully, moisten with $CaCl_2$ solution; evaporate again, *a rosy-red spot = inosit.*)

9. If not a proteid and not soluble in cold water, apply heat:

SOLUBLE.	INSOLUBLE.	MELT.
Starch.	*Uric acid.*	*Fats* (apply ether test).
Tyrosin.	*Cholesterin.*	
Urates.		

If soluble, to a portion of the watery solution apply the test for *starch*. If no result, test for *tyrosin* (see 3), and for *urates* (p. 189).

10. If not a proteid and insoluble in water (cold or hot), apply murexide test for *uric acid*.

11. If no result, test for *cholesterin*.

PART III.

PRACTICAL PHYSIOLOGY.

CHAPTER I.

EXAMINATION OF THE CIRCULATION MICROSCOPICALLY.

THE circulation may be studied (*a*) in the web of a frog's foot (this is the most easy method, as a frog can readily be obtained at all seasons of the year); (*b*) in the tail of a tadpole; (*c*) in the caudal fin of a small fish, *e.g.*, goldfish or minnow; (*d*) in the mesentery of any of the smaller mammals, such as the mouse or young rat.

To demonstrate the circulation in the web of a frog's foot, prepare a small stand upon which to rest the body of the animal. Such a stand is readily made from a block, or by supporting a thin and flat piece of wood upon props by the side of the microscope stage. In the latter case a hole with a diameter of about three-fifths of an inch should be made near one extremity. At the end of the board nearest the hole, slits are to be cut for the passage of threads. A light-coloured frog is then to be selected, and its head wrapped in a damp cloth, whilst its body is arranged in such a manner upon the stand, that one hind-foot extends over the hole in the board, the other being tucked up out of the way. Ligatures should then be passed over the ends of two adjoining toes and pulled tight. In this way, by a little mani-

pulation, the threads may be fixed so as to allow of a flat surface of the web between the toes being satisfactorily examined. Care must be taken not to stretch the web to an excessive extent, lest the circulation be impeded. In the majority of cases, the frog will remain perfectly quiet for a long period of time, and this is especially the case if the nose of the animal be brought into close contact with the board upon which it lies. Occasionally the frog resists all blandishments of this nature, and exhibits the greatest restlessness. It will then be necessary to subject it to the influence of ether, or to inject beneath the skin of the back a very weak aqueous solution of urari. The effect of the drug is to render the animal motionless, by paralyzing the endings of the nerves in muscles, and thus preventing the transmission of motor impulses. Urari requires from fifteen to thirty minutes to produce its full effect. The web, after a suitable piece has been obtained for examination, should be brought into focus, and should be examined first with a low power of the microscope, and afterwards with a high power. From time to time, during the examination, the web should be moistened with water. The examination should not be commenced until two or three minutes after the web has been fixed, in order to allow the circulation, as far as possible, to return to its normal condition.

Under the low power notice and draw (*a*) the black *pigment cells* of irregular shape and of varying size lying more superficial than (*b*) *the arterioles*, in which the blood current is more rapid than in (*c*) *the venules*, (*d*) *the capillaries*. Observe the alterations in their size. With the low power select a thin piece of the web, where the vessels can be distinctly seen, and continue the observation under the high power. Determine (*a*) the relative positions of the coloured and colourless corpuscles, the coloured in the centre, the colourless at the sides of the vessels; (*b*) the diapedesis, or passage of colourless blood corpuscles through the walls of the capillaries. This phenomenon can, however, rarely be observed.

The circulation in the tail of the tadpole is readily examined if the animal be first placed in a watch-glass full of water, to which a drop or two of urari solution has been added. When

the tadpole has become motionless, it should be transferred to a slide, and examined at leisure. If it is considered necessary, the thinner portions of the tail may be covered with a cover-glass. The same features will be recognised as were described in the case of the frog's web.

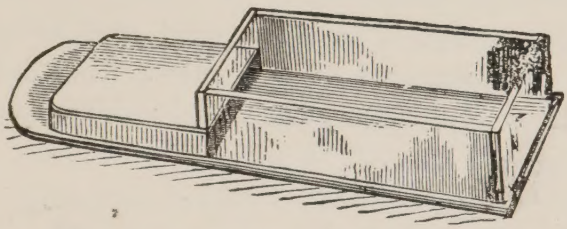

Fig. 26—Glass for examining the Circu ation in the Fish's Tail.

For the examination of the circulation in a fish, all that is required is to place the fish, generally a goldfish, in a suitable vessel, through which a stream of water is kept running continuously. This can be done by means of Caton's trough (Fig. 26), or in a simple glass box partially covered over. Into the covered part the tail is inserted, the fish lying comfortably in the trough, which is filled with water, and into which a constant stream flows. The box is placed upon the stage of the microscope, and the tail can be examined with a low power.

The arterial schema is an apparatus designed to represent in a diagrammatic form the main phenomena of the arterial pulse. It consists of a series of elastic tubes of varying calibre, which are arranged somewhat as they are in the vascular system, the larger tubes being equivalent to the main vessels, the smaller ones to the arterioles and venules. The ends of the tubes can be closed by means of ivory pegs, and in this way and by clamps the resistance within the system can be raised to any required extent, and the varying conditions of the circulation can be imitated. In the centre of the system is a thick-walled elastic sac provided with valves, as in the case of an enema syringe, so that fluid can pass through it in one direction only. When required for use, the tubes are moderately distended with water. Three levers similar to those employed with the

sphygmograph are arranged at intervals of six to eight inches along the tubes, in such a way that their movements are recorded in a vertical series of tracings upon the revolving drum. The central sac is then compressed rhythmically by the hand, and the resulting tracings made by the levers are afterwards carefully noted and compared.

The sphygmograph is an instrument used for representing graphically the characters of the pulse. In it a small button rests upon the artery, usually the radial ; this button is attached

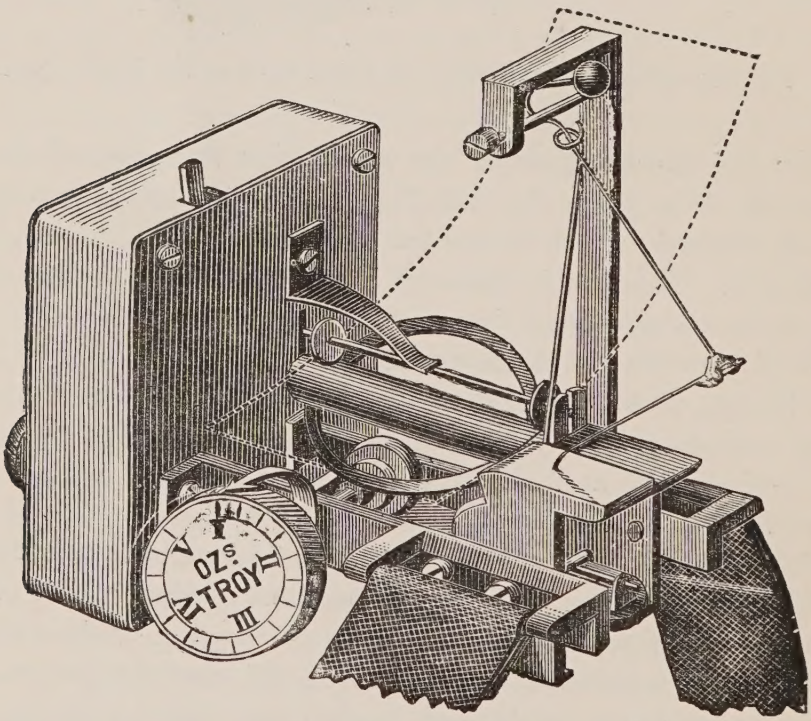

Fig. 27—Dudgeon's Sphygmograph.

to the under surface of a steel spring. The movement of the button is communicated to an upright screw working in an arm of metal, which, with the spring, is fixed (although capable of up and down movement) by means of a screw to the frame of the apparatus. The movement of this adjustable screw is communicated to a lever of light wood, since the metal arm has in front a piece of metal projecting upwards, which comes in

contact with the lever near its fulcrum. The lever writes on a smoked glass or card, which is moved by clockwork along a groove on the upper surface of the brass box containing the clockwork, which is fixed on the flat piece of metal forming the frame of the instrument. The sphygmograph is bound on to the wrist with the button on the artery (but not pressing too hard), and the clockwork backwards. The smoked surface is arranged in place ; the lever adjusted by means of the screw, so that its end writes on the smoked surface by means of a sharp point. The clockwork is wound up and set going, and the character of the pulse is represented on the moving surface by means of a tracing on the paper. The clockwork is then stopped, the tracing removed, the circumstances under which it was taken noted down, and the paper or glass is varnished. Nearly all the best sphygmographs are provided with an apparatus for adjusting the amount of the pressure which the spring exercises upon the artery. The form of the instrument chiefly used in this country is a modification of the original sphygmograph of M. Marey.

A small instrument invented by Dudgeon is in considerable use ; it is represented in Fig. 27. The advantages claimed for it are : It magnifies the movements of the artery in a uniform degree, viz., fifty times. The pressure of the spring can be regulated from one to five ounces. It requires no wrist-rest, and may be used with equal facility whether the patient is standing, sitting or lying. With it a tracing of the pulse can be made almost as quickly as the pulse can be felt with the finger. Its sensitiveness is so great that it records the slightest deviation in the form or character of every beat. Its construction is so simple, that if accidentally broken any watchmaker can repair it. It is so small ($2\frac{1}{2}$ by 2 inches), and it is so light (4 ounces), that it can easily be carried in the pocket. It is only one-third of the price of the ordinary instruments.

Method for estimating the Blood Pressure in a Rabbit.*— Make a saturated solution of chloral hydrate in ·75 per cent. saline solution ; inject 15—20 minims of the chloral solution

* A license under the Vivisection Act is necessary before this experiment can be legally performed.

beneath the skin of the abdomen of a live rabbit. Leave the rabbit for half an hour, and in the interval prepare the rest of the apparatus. Fill a pressure-bottle with a saturated solution of sodium sulphate, and suspend it by means of a string and pulley attached to the ceiling, about four feet above the operating-table. The bottle should have a hole near its lower part, to which a long india-rubber tube is attached : the fluid is prevented from running out by means of a clamp attached to the lower part of the tube. Get ready Czermak's rabbit-holder, and arrange ligatures upon it for binding the fore and hind legs of the animal. Arrange the recording apparatus (p. 228) on one side of the rabbit-holder, and see that the clockwork is wound up, and that it is in working order. Gum a slip of glazed paper round the recording drum (p. 229), and blacken it by revolving the drum over the flame of a paraffin lamp. The coating of lamp-black should be as uniform as possible, and not too thick. If the kymograph writes with a pencil or with a pen, such blackening is not requisite ; in the latter case fill the can of the pen with a few drops of aniline ink. If a continuous tracing is to be taken, arrange the feeding-roller in a proper position. In the case of the mercurial, or Ludwig's kymograph, see that the U tube is partially filled with clean and bright mercury ; that the top of the float is not below the level of the mercury, and that the weighted thread presses upon one arm of the pen in such a way as to keep it in contact with the drum. Block up the shorter limb of the U tube with a piece of wood. Take a T-shaped glass tube, connect the vertical portion with the tube descending from the pressure-bottle ; and one of the horizontal rami with the tube which projects from the shorter limb of the U tube, by means of a piece of india-rubber tubing (or better still, by means of a piece of substantial leaden piping). To the other horizontal ramus attach a piece of india-rubber tubing, and clamp the end ; open the clamp which has hitherto closed the tube leading from the pressure-bottle ; the sodium sulphate will pass through the T-shaped tube, and will fill the tubes in connection with it. Remove the piece of wood from the top of the shorter limb of the U tube, and replace it as soon as all the air is expelled, and the limb is full of sodium sulphate solution. Loosen the clip

upon the india-rubber tube for an instant, and allow a few drops of the solution to escape ; no air should now be present in the system of tubes thus arranged : if leakage takes place in any part, and air enters, it must be remedied. Select or make a cannula large enough for the carotid or femoral artery of the animal to be experimented upon ; this requires considerable experience, and reference had better be made to the demonstrator. Arrange the instruments near at hand which are requisite for the operation of exposing the vessel : they are a box of scalpels, scissors, and forceps, fine and coarse, a pair of each ; a pair of Krönecker's bull-dog forceps, aneurysm needle, ligatures of silk, a splinter of wood (a match answers the purpose very well), a 'seeker,' and sponges.

Fix the rabbit upon the rabbit-holder ; it should be insensible at the time of the operation, and is fixed by laying it upon its back, unscrewing the bit at the end, and removing the central steel peg ; pass the peg through the side of the bit, in such a way that it passes within the mouth, between the lips and behind the incisors of the two jaws, passing out on the opposite side, where it fits into a hole in the bit. The animal is thus held by the teeth. Screw up the end of the bit ; the lower jaw will thus be compressed, and the animal will thereby be prevented from opening its mouth and loosening the central pin ; if need be, the pressure may be increased from time to time by means of the screw. Extend the hind-legs, and pass the ligatures over the thigh on each side ; tighten and fix them. Pass ligatures over the fore-legs, and fix them in such a way that the first joints are flexed, the limb being held at the elbow.

Before proceeding further, see that everything is in proper working order. The points to be chiefly attended to are : (1) *The recording apparatus*, that it is wound up, and that it works smoothly. (2) *The kymograph*, that the pen or style writes distinctly, and without too much pressure ; that no bubbles of air exist in the tube. (3) *The system of tubes*, that they are full of the solution of the sulphate of soda, that they contain no air, and that they are not kinked. (4) That *the pressure bottle* is neither too high nor too low ; and that if need be it can readily be raised or lowered to equalize the pressure of blood. (5) That

the distal end of *the cannula* fits accurately, and can be easily tied into the proximal clamped end of the india-rubber tubing which is in connection with the pressure-bottle and the kymograph.

Expose the carotid in the rabbit. First clip away the fur over one side of the neck, and make an incision along the side of the trachea for about three inches. Separate the muscles carefully with a seeker, taking care not to go too far outwards. The artery is readily discovered; it may be distinguished from the large vein which accompanies it by its more opaque appearance, as well as by its lighter colour, and by the pulsation which it exhibits. Dissect it out carefully for a short distance, and pass the aneurysm needle, armed with a ligature beneath it; withdraw the needle, leaving a loop of thread round the artery; cut the loop and two ligatures will be thus formed. With the bull-dog forceps, clamp the proximal portion—*i.e.*, the part nearest the heart—of the artery. With one of the ligatures tie the distal portion of the artery as high up as the incision will allow. Raise the portion of the artery between the clamp and the ligature by passing the splinter of wood beneath it, and with a pair of sharp and fine-pointed scissors make a V-shaped incision into it. Fill the cannula, by means of a pipette, with the sodium sulphate solution, and insert it into the incision in the artery; the smaller end being directed towards the heart. Pass the second ligature over the cannula, and tighten it round the artery in such a way as to tie the cannula firmly into the vessel (to facilitate this operation the cannula is provided with a shoulder, behind which the ligature should pass). Fit the india-rubber tube leading through the T piece to the manometer, on to the other end of the cannula, taking care that both the tube and the cannula are quite full of the soda solution, so that no air may be enclosed at the point of junction. Remove the clamp which has hitherto prevented the escape of fluid from the india-rubber tube nearest the cannula. Set the clockwork of the recording apparatus in motion; with one hand open *cautiously and gradually* the clamp upon the tube of the pressure-bottle, whilst with the other hand remove the bull-dog forceps from the artery. If everything has been properly arranged, a few drops

of blood will pass into the cannula, but the pressure in the artery will be counteracted by the column of sodium sulphate solution, which transmits the variations in the blood pressure to the mercury in the U tube, whence it is transmitted to the lever which records it upon the drum. It is possible that the pressure bottle may be too high, and that the column of liquid is more than enough to counterbalance the blood pressure; in which case the sulphate of soda will enter the blood, and will not only vitiate the experiment, but in many cases will actually kill the animal; hence it is necessary to be cautious in opening the clamp. The sulphate of soda arrests, to a certain extent, the coagulation of the blood, and it is therefore employed; but it often happens that the cannula becomes blocked by a clot, in which case it will be necessary to detach the tubing and clean out the cannula, or it may be necessary to clamp the artery, and insert a fresh cannula.

To make a Cannula for use during Blood-pressure Experiments.—Take a piece of hard glass tubing, with a bore of about the size of an ordinary quill pen. Soften the end of the tube in the flame of a blow-pipe, and draw it out gently for about an inch: there will then be a narrower portion of tube between two pieces of the full size. When the tube has cooled, heat a portion of the narrower part in the flame, and draw it out very slightly; by this means the narrower portion will be thicker in the centre than at one side. File through the middle of the narrower portion in an oblique direction. A cannula with an oblique opening at its smaller extremity will thus be formed. It must be finished by carefully rounding off its edges in an ordinary gas flame, and by filing down the aperture with a three-cornered file, until it presents the necessary obliquity. The narrowed portion will have a neck to prevent the ligature slipping off when it is tied into the artery.

The cardiograph registers in a graphic manner the heart's impulse. It consists of two portions: (*a*) A hollow metal disc whose face is covered with a thin membrane of india-rubber. The disc is provided with three levelling screws; from its posterior surface passes off a tube bent at right angles. In front of the elastic membrane is an ivory knob, which is in connection

with a delicate spring arising from the side of the disc. The extremity of the spring is also provided with a pointed steel screw, resting exactly on the centre of the membrane. (*b*) The registering portion (Marey's tambour) consists of a second disc, whose elastic membrane is in connection with a lever, and from whose under-surface a tube also proceeds. The tubes of the two discs are connected with each other by a portion of elastic tubing, and in this way an air-tight cavity is produced, so that any movements executed by the membrane of the first disc are reproduced in the second disc, and are transmitted to the lever by which they are recorded in the ordinary way upon the rotating drum. In using the instrument, the patient is made to lie down upon a couch of convenient height ; the chest is bared, and the apex beat of the heart is found in the fifth left costal interspace, somewhat below and internal to the nipple. The first disc is then applied in such a way that the ivory knob is exactly over the point at which the beat of the heart is felt. The impulse is thus transmitted to the lever, which executes certain movements. If these movements be registered upon the revolving drum, it will be found that they consist of a sudden ascent at the instant of the ventricular contraction, and of an equally marked but less sudden fall.

The Stethoscope.—The sounds of the heart are heard by means of the stethoscope. The simplest form of this instrument is a cylinder of wood or metal expanded at one end into a conical portion, which is applied to the chest wall, whilst the opposite extremity is provided with a slightly concave disc, to adapt it to the observer's ear. The room should be perfectly quiet, the patient should bare his chest, and remain standing. The finger should then be placed upon the apex-beat of the heart. The observer, standing in front of the patient, should apply the conical end of the stethoscope over this point, and his ear to the opposite end : at the same time he should feel with the fingers of his left hand beneath the sterno-mastoid muscle of the left side for the carotid artery. Two sounds will then be heard, one accompanying the impulse, called the *first* or systolic sound ; the other following the impulse, and known as the *second*, or diastolic sound. The first sound is the longer and more deep-

toned ; it is best heard at the apex of the heart. The second sound is sharper and shorter ; it is best heard in the third intercostal space, close to the sternum, though it is also audible at the apex. After the second sound is a pause, so that the normal cardiac cycle is roughly represented by the rhythm *lubb, dŭp—lubb, dŭp.* In listening to the sounds of the heart, the respiratory sounds may be neglected. Care must be taken that the stethoscope be applied evenly to the chest wall, that the tube is not touched by the clothes or fingers whilst the examination is being made, and that the observer does not press so heavily against the stethoscope as to cause pain to the patient.

The ophthalmoscope, brought into use by Helmholtz, consists in its simplest form of (*a*) a slightly concave mirror of metal or silvered glass, perforated in the centre, and fixed into a handle ; and (*b*) a biconvex lens of about $2\frac{1}{2}$—3 inches focal length. Two methods of examining the eye with this instrument are in common use—the direct and the indirect ; the student should endeavour to accustom himself to use both methods of investigation with equal facility.

A normal eye should be examined ; a drop of a solution of atropine* (two grains to the ounce) should be instilled about twenty minutes before the examination is commenced ; the ciliary muscle is thereby paralyzed, the power of accommodation is abolished, and the pupil is dilated. This will materially facilitate the examination ; but it is quite possible to observe all the details to be presently described without the use of this drug. The room being now darkened, the observer seats himself in front of the person whose eye he is about to examine, placing himself upon a somewhat higher level. A brilliant and steady light is placed close to the left ear of the patient. The atropia having been put into the right eye *only* of the patient, this eye is examined. Taking the mirror in his right hand, and looking through the central hole, the operator directs a beam of light into the eye of the patient. A red glare, known as *the reflex*, is seen ; it is due to the illumination of the retina. The patient is then told to look at the little finger of the observer's

* Or preferably homatropin hydrobromate, as the effect passes off more rapidly.

right hand as he holds the mirror ; to effect this the eye is rotated somewhat inwards, and at the same time the reflex changes from red to a lighter colour, owing to the reflection from the optic disc. The observer now approximates the mirror, and with it his eye to the eye of the patient, taking care to keep the light fixed upon the pupil, so as not to lose the reflex. At a certain point, which varies with different eyes, but is usually when there is an interval of about two or three inches between the observed and the observing eye, *the vessels of the retina* will become visible as lines running in different directions. Distinguish the smaller and brighter red arteries from the larger and darker coloured veins. Examine carefully the fundus of the eye, *i.e.*, the red surface—until *the optic disc* is seen ; trace its circular outline, and observe the small central white spot, the *physiological pit :* near the centre is the central artery of the retina breaking up upon the disc into branches ; veins also are present, and correspond roughly to the course of the arteries. Trace the vessels over the disc on to the retina. The optic disc is bounded by two delicate rings, the more external being the choroidal, whilst the more internal is the sclerotic opening. Somewhat to the outer side, and only visible after some practice, is the *yellow spot*, with the small lighter-coloured *fovea centralis*, in its centre. This constitutes the direct method of examination ; by it the various details of the fundus are seen as they really exist, and it is this method which should be adopted for ordinary use.

If the observer is ametropic, *i.e.*, is myopic or hypermetropic, he will be unable to employ the direct method of examination until he has remedied his defective vision by the use of proper glasses.

In the indirect method the patient is placed as before, and the observer holds the mirror in his right hand at a distance of twelve to eighteen inches from the patient's right eye. At the same time he rests his little finger lightly upon the temple, and holding the lens between his thumb and forefinger, two or three inches in front of the patient's eye, directs the light through the lens into the eye. The red reflex, and subsequently the white one, having been gained, the observer slowly moves his

mirror, and with it his eye, towards or away from the face of the patient, until the outline of one of the retinal vessels becomes visible, when very slight movements on the part of the observer will suffice to bring into view the details of the fundus above described, but the image will be an inverted one. The lens should be kept fixed at a distance of two to three inches, the mirror being alone moved until the disc becomes visible ; should the image of the mirror, however, obscure the disc, the lens may be slightly tilted.

The laryngoscope is an instrument employed in investigating during life the condition of the pharynx, larynx, and trachea. It consists of a large concave mirror with perforated centre, and of a smaller mirror fixed in a long handle. It is thus used : the patient is placed in a chair, a good light (argand burner, or lamp) is arranged on one side of, and a little above his head. The operator fixes the large mirror round his head in such a manner that he looks through the central aperture with one eye. He then seats himself opposite the patient, and so alters the position of the mirror, which is for this purpose provided with a ball and socket joint, that a beam of light is reflected on to the lips of the patient.

The patient is now directed to throw his head slightly backwards, and to open his mouth ; the reflection from the mirror lights up the cavity of the mouth, and by a little alteration of the distance between the operator and the patient the point at which the greatest amount of light is reflected by the mirror—in other words its focal length—is readily discovered. The small mirror fixed in the handle is then warmed, either by holding it over the lamp or by putting it into a vessel of warm water ; this is necessary to prevent the condensation of breath upon its surface. The degree of heat is regulated by applying the back of the mirror to the hand or cheek, when it should feel warm without being painful.

After these preliminaries the patient is directed to put out his tongue, which is held by the left hand gently but firmly against the lower teeth, by means of a handkerchief. The warm mirror is passed to the back of the mouth, until it rests upon and slightly raises the base of the uvula, and at the same time the

light is directed upon it : an inverted image of the larynx and trachea will be seen in the mirror. If the dorsum of the tongue be alone seen, the handle of the mirror must be slightly lowered until the larynx comes into view ; care should be taken, however, not to move the mirror upon the uvula, as it excites retching. The observation should not be prolonged, but should rather be repeated at short intervals.

The structures seen will vary somewhat according to the condition of the parts as to inspiration, expiration, phonation, etc. ; they are first, and apparently at the posterior part, the *base of the tongue*, immediately below which is the arcuate outline of the *epiglottis*, with its cushion or tubercle. Then are seen in the central line the *true vocal cords*, white and shining in their normal condition. The cords approximate (in the inverted image) posteriorly ; between them is left a chink, narrow whilst a high note is being sung, wide during a deep inspiration. On each side of the true vocal cords, and on a higher level, are the pink *false vocal cords*. Still more externally than the false vocal cords is the *aryteno-epiglottidean* fold, in which are situated upon each side three small elevations ; of these the most external is the *cartilage of Wrisberg*, the intermediate is the *cartilage of Santorini*, whilst the summit of the *arytenoid cartilage* is in front and somewhat below the preceding, being only seen during deep inspiration. The *rings of the trachea*, and even the bifurcation of the trachea itself, if the patient be directed to draw a deep breath, may be seen in the interval between the true vocal cords.

CHAPTER II.

NERVE MUSCLE PHYSIOLOGY.

CONTRACTILITY OF MUSCLE.

In order to show the excitability of living muscle it is usual to employ the muscles of a pithed frog, either remaining *in situ* or removed from the body. Any muscle will answer the purpose which may be conveniently dissected out, but it is usual to employ those to which nerves can be easily traced, and to apply the stimuli to the nerve supplying the muscle, instead of to the muscle itself directly.

The first operation required is *to pith a frog, i.e.*, to completely destroy its central nervous system. In order to accomplish this the animal must be held by its two fore-legs in such a way that its belly is pressed against the dorsal surface of the left index finger, the head projecting beyond the tip of the finger, and being pressed downwards by the thumb of the left hand. The skin on the back is then put on the stretch, and the nail of the right index finger is drawn down the centre of the head towards the spinal column. Immediately below the head, and at its junction with the vertebræ, a slight depression will be felt, marking the position of the occipito-atlantoid membrane. A small triangular snip is made over this spot with a pair of sharp-pointed scissors, the membrane is then divided, and a wooden match sharpened at one extremity is thrust upwards into the brain to destroy sensibility, and then a long blanket-pin is pushed downwards into the spinal canal to destroy the spinal cord. In performing this operation no bleeding should occur. Care must be taken that

the pin really enters the spinal canal, and that on the one hand it does not simply pass beneath the skin of the back, whilst on the other it does not pass into the abdominal cavity. The sudden extension of the hind limbs may be taken as a proof that the destruction of the cord has been properly completed.

A Nerve-Muscle Preparation may be made in this manner:

(i.) Having pithed a vigorous frog, open the abdomen with a pair of sharp scissors, turn aside and remove the viscera, and expose the sacral plexus of each side ; free the nerves from connective tissue, clamp the head of a frog in a holder, and attach to a retort stand, allowing the lower extremities to hang about eight or ten inches above the table. A pair of electrodes may now be inserted behind the nerves of the plexus. The electrodes are made by fixing with sealing-wax two pieces of copper wire in glass tubes about an inch and a half long, and allowing the ends to project, fastening the tubes together with sealing-wax, and soldering to one end of the projecting wires two thinner flexible wires, two feet long, covered with cotton. The ends projecting not quite so far at the opposite ends of the tubes are then scraped with a knife, and bent nearly at right angles, and the electrodes are complete. Convenient electrodes may also be made by inserting pins two inches long into a cork, soldering wires to the heads, and turning up the points as above.

(ii.) The directions for making the more usual form of nerve-muscle preparation, however, are as follows : Pith a frog and remove the skin from the back of one thigh ; this will expose the muscles enclosed in a fine sheath of fascia ; with two pairs of forceps tear the muscles apart by breaking open the sheath. Three muscles will be exposed—the *triceps*, on the outside, the *semi-membranosus* on the inside, and lying between the two, and partially covered by them, a smaller muscle with tendinous ends, the *biceps*. Follow the biceps to its origin, and carefully cut it through with a pair of scissors ; then catching hold of the divided end, pull it forcibly down towards its insertion, and entirely remove it. By this method of procedure the sciatic nerve will have been exposed. Carefully dissect it out, tracing it upwards to spinal column, and downwards to the gastrocnemius—notice its first and chief division to supply the two heads ; do

not prick the bloodvessels in contact with the nerve, but gently separate them : cut through the spinal column with spinal cord, and divide the part detached vertically into two, and by holding the piece of bone belonging to the side you are dissecting, lift up the nerve, and free it from surrounding tissues. The nerve will now be ready for succeeding experiments. During the operation it ought not to have been pinched, pricked, or otherwise injured.

The nerve must be placed upon the electrodes, and the preparation is ready for the demonstrations of muscular contractions under appropriate stimuli. Care should be taken that the preparation, whichever it be, should not dry up.

Stimuli are either (1) *electrical*, (2) *mechanical*, (3) *thermal*, or (4) *chemical*.

(1) Of *electrical stimuli*, the effects of (*a*) the *induction shock* and (*b*) the *galvanic current* must be demonstrated.

(*a*) **Effects of the Induction Shock.**—Apparatus required :

(i.) A *battery*, the one in most common use being *Daniell's*, which consists of an outer vessel of copper, which forms the negative plate, full of a saturated solution of copper sulphate in which is placed a porous earthenware vessel containing a stout rod of well-amalgamated zinc, which forms the positive plate, immersed in dilute sulphuric acid (1 in 8). The electrical current passes from the copper to the zinc outside the battery, and in the reverse direction inside. The electrode attached to the copper is the positive, and that to the zinc the negative—the copper being the positive pole and the zinc the negative. From the presence of the two fluids the current of electricity is pretty constant in strength, altering little during long periods of time, hence called *constant* or *cont'nuous*. A supply of solid copper sulphate is placed upon a ledge attached to the inner and upper part of the copper vessel, so that the solution of copper sulphate may be maintained at the point of saturation.

(ii.) *Two keys*, one of which should be of the form indicated in Fig. 28, by means of which the current may be made or interrupted, and the other a mercury key.

(iii.) *Wires*, insulated by a gutta-percha covering.

(iv.) *Electrodes.*—Either the glass or cork form mentioned above. A pair with platinum points would be useful.

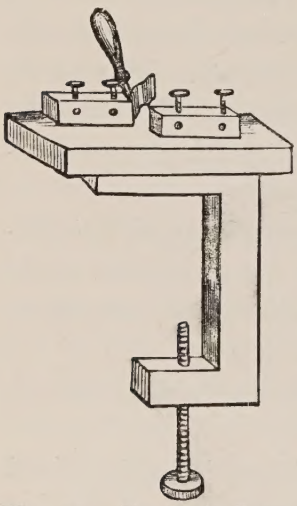

Fig. 28. Du Bois Reymond's Key.

(v.) Du Bois Reymond's induction apparatus (Fig. 29).

Method of Experiment.—(i.) Prepare a Daniell's cell, connect it by wires with the top screws (*d d*) of an induction coil (Fig. 29)—these screws are in connection with the ends of the coil of wire forming the *primary coil* (*c*)—interposing a mercury key, attach electrode wires to screws of secondary coil (*g*), interposing a key (Fig. 28) for short circuiting, place electrodes behind nerve of a nerve-muscle preparation of the second form indicated above. Each time the battery circuit is completed by closing the key, and broken by opening the key, an induced current is momentarily produced in the secondary coil in opposite direction

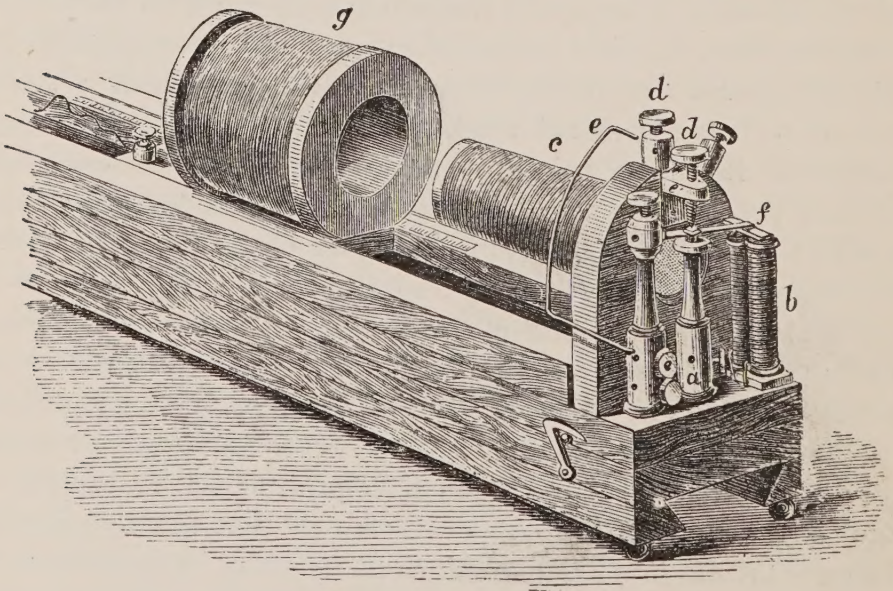

Fig. 29. Du Bois Reymond's Induction Coil.

on making, and in the same direction on breaking; and each of these, if strong enough, will produce a single muscular contraction.

(1) *To show that the breaking contraction appears before the making.*—Slide the secondary coil a considerable distance from the primary coil, open and shut mercury key—no contraction. Move secondary coil nearer primary coil until a contraction ensues—note that contraction first appears when key is opened (*breaking contraction*)—note the distance in millimetres—proceed until a contraction appears also on closing key (*making contraction*).

(2) *To show that the stimulus is increased as it descends the nerve.*—In a preparation in which the sciatic nerve is dissected out, but not cut, proceeding in an exactly similar way to (1), note results (in form of a table) with platinum electrodes placed behind nerve, (*a*) high up, (*b*) mid-way, (*c*) close to muscle, and also (*d*) with electrodes upon gastrocnemius itself.

(3) *To show that when the nerve is divided, its irritability is at first increased, in the neighbourhood of the section.*—Divide the nerve high up, and repeat experiments ; compare results.

(4) *To show the effect of temperature upon irritability.*—In preparation which has been kept cold in ice, and (5) In another, which has been warmed moderately, repeat the above experiments.

(6) *To show that irritability gradually diminishes some time after section of the nerve has been done.*—If time permit, repeat experiment (2) with the same preparation after an hour has elapsed, and again after a second hour, and compare results.

N.B.—Always be careful that your battery is working, your wires unbroken, your contacts secure, and your metallic connections bright.

The use of the key in the secondary circuit is to cut off the make or break contraction, or both ; thus if key 2 is open whilst key 1 is opened and shut, contractions will occur at make and break. If key 2 is open, and then key 1 is opened, a breaking contraction will occur, but the making contraction may be cut off by closing key 2 before key 1. And similarly, if key 2 is closed and then key 1 is opened, and opened before key 1 is shut, a single making contraction will occur. If key 2 is closed, whilst key 1 is opened and shut, no contraction will occur.

Effects of a Series of Induction Shocks.—Connect the wires from the battery with the screws at the bases of the two pillars of an induction coil (*c*, *a*). Interpose a key in the secondary circuit. The current passes up the outer pillar, along the spring, until it arrives at the point where contact is made by means of a platinum-pointed screw (*e'*), adjustable, and in connection with the

end of the primary coil ; the current passes by this connection through the primary coil, and then through the coils of wire surrounding two pieces of soft iron, and thence to the battery through the middle pillar coil. As soon as the current passes through the electro-magnet, the soft iron is magnetized, and draws down the hammer. This breaks contact with the spring, and the current is by this means interrupted, to be again made as soon as the magnet ceases to act from the stoppage of the current through the electric coil round its soft iron. In this way a series of rapid make and break shocks occurs, and these are represented in the induced current, and therefore in a series of make and break contractions of the muscle, when the electrodes

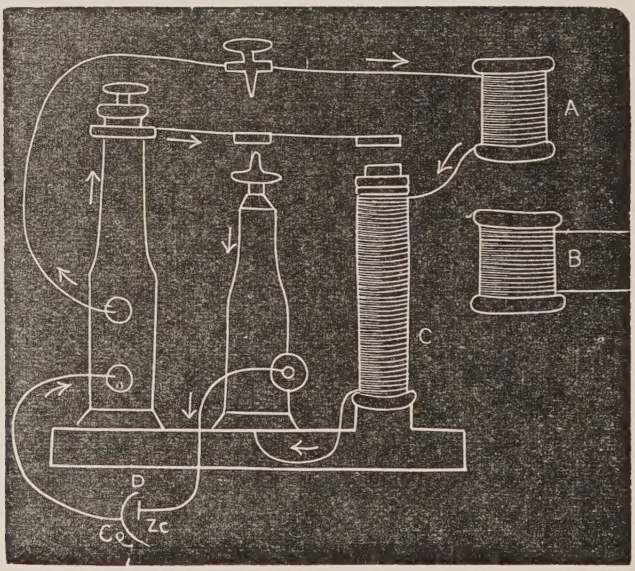

Fig. 30. Diagram of the Course of the Current through the Induction Coil when Helmholtz's Connecting Wire is used.

placed under the nerve are connected with the screws of the secondary coil, and the key is opened ; the effect of this is to send the muscle into a state of tonic spasm, or *tetanus*. The frog's leg and foot are rigidly extended. The contraction will continue for some time, but will finally give way under prolonged stimulation.

The apparatus is known as the *magnetic interrupter*. With the magnetic interrupter thus employed, the break current is found to be much more powerful than the make, and so the

stimuli are not uniform, as they should be to produce a true tetanus. A modification, therefore, is necessary, the object of which is to equalize, as far as possible, make and break shocks. This consists of a stout copper wire to connect the outside pillar with a binding screw, which is in connection with the screw at one end of the primary coil; in this way part of the current always passes at once to the primary coil and only a portion of it is made and broken by the interrupter, whether or not the plate is attracted to the magnet. The point of the middle pillar must be raised by means of the middle screw, and the screw attached to the end of the primary coil must also be screwed up away from contact with spring. The current on entering (supposing the contact between the spring and the middle pillar to be made) divides; one portion passes through the primary circuit and magnet, the other passes through the contact down the middle pillar and back to the battery (Fig. 30); but as the part of the current which passes through the magnet is insufficient for the purpose of retaining the hammer in contact, the current is broken by the hammer springing away from the magnet; then the whole of the battery current passes through the primary coil and magnet, and again the hammer is attracted to the latter, and so on. It will be seen that by this arrangement only a portion of the current is interrupted.

If time permit, the following further experiments may be done before proceeding to (*b*):

The extra current of Faraday may be demonstrated by taking a Daniell's element, two keys, the primary coil, a nerve-muscle preparation, and electrodes. The apparatus is arranged so that both keys, as well as the induction coil, are placed in the primary circuit, whilst to the second key the electrodes are connected. On testing the current with the tongue, supposing the key No. 1 be open, on opening key No. 2, as well as on shutting it, there is an appreciable effect upon the tongue. When the coil is cut off by closing key 1, there is very little or no effect on opening key 2. The effect is produced by the extra induced current. It may also be shown by diminishing the battery current by a wire directly connecting the poles until no contraction occurs. If key 1 is closed, then, on breaking with the primary coil, a contraction will occur.

Unipolar Excitation.—Arrange the battery and coil for single induction shocks, and connect one electrode with one of the screws at the end of the secondary coil. Place this under the exposed sciatic nerve of a nerve-muscle preparation, which should be arranged on a plate of glass upon a

frog board; *i.e.*, a flat piece of board covered with cork. Open and shut a key in the primary circuit, and there will be no response; now touch the muscle with the finger or a pair of forceps held in the hand, and it will contract.

Galvani's Experiment.—Take a piece of zinc, thoroughly cleaned, and coil round one end a piece of copper wire, which projects in such a way that a fork with two equal prongs is made. Insert the zinc behind the lumbar nerves of a pithed frog, and allow the copper to fall upon the thigh muscles; a contraction will occur. If the muscles be very excitable there will also bea contraction when the copper is removed from the muscle.

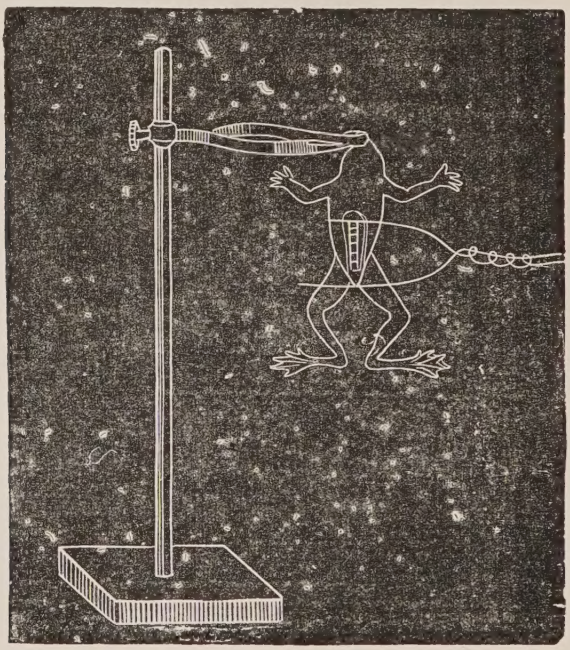

Fig. 31. Galvani's Experiment.

Interruption by means of a Spring.—Bring one of the wires from a Daniell to the end of a steel spring which is fixed in a support in such a way that when it is set into vibration the opposite end dips into a mercury cup in metallic connection with the primary coil, and which is also connected with the battery (the spring and cup taking the place of a key), connect the electrodes with the secondary coil, and place them under the sciatic nerve of a pithed frog. Make the spring vibrate, and notice that if the spring is short, tetanus is at once set up, and if long, that one contraction is distinctly piled on the top of another at first, and that a more gradual tetanus is produced.

Interruption by means of a Metronome.—Insert a vibrating metronome into the primary circuit of an induction coil, and allow the series of induction shocks thus regulated to break into a nerve-muscle preparation. The limb will gradually pass into tetanus.

(*b*) **Effects of the Galvanic Current.**—Prepare a nerve-muscle preparation, and get ready a Daniell's cell. Bring the wires of the latter to the inner binding screws of a Du Bois

Reymond's key (Fig. 28). Bring the electrode wires to the outer binding screws ; arrange the electrodes behind the nerve of the preparation. Open the key by raising the handle, and after a few seconds close it again ; a contraction of the muscle will occur at the opening and at the closing, that is to say, at the make and at the break, and *during the interval there will be no contraction*, although the current is passing through the nerve all the time. The use of the key is to shut off the current from the nerve, as whilst it is closed the brass plate affords much less resistance to the current than the nerve ; and as the current will pass in the direction of least resistance, the whole of it passes through the key to the battery again. On opening the key the current is bound to pass through the nerve, which it excites, and so produces contraction in the muscles supplied by it. The electrode connected with the copper, or the *positive electrode*, is called the *anode*, and that connected with the zinc, or the *negative electrode*, is called the *kathode*. As the current passes from the positive to the negative, when the kathode is nearer the muscle than the anode, the current is said to be *descending ;* and when, on the other hand, the kathode is above the anode, the current is called *ascending*.

If the key be *opened* for some time, and then the battery wire be removed, a contraction will occur several times on closing the key, without the battery current ; this is due to the *polarization of the electrodes* by the current : as many as twenty contractions may be shown under favourable circumstances in this way.

Regulation of the Strength of a Galvanic Current. The Rheochord.—This is done by interposing in the circuit a graduated wire, the resistance of which is regulated. This wire is arranged to form the *rheochord* (Fig. 32). The one in use is that of Du Bois Reymond.

The instrument consists of a long box or board, on which the resistance wires are stretched. At one end are fixed several brass blocks, separated from one another, and disconnected except by fine German-silver wire. At each corner of this end are binding screws, A and B, each connected with the brass block nearest. Beginning at the block nearest A, a wire passes a considerable distance up the board, passes round a peg and returns

to the second block, from which a wire, in a similar manner, considerably shorter, connects it with the next block, and so on. From the two blocks at the end of the row two thin platinum wires pass to the opposite end of the board (C), and are there insulated ; they are, however, connected by means of a slide, formed of cups of mercury. This slide is capable of being moved up and down the wires. The brass blocks are, moreover, capable of direct connection (except the two at the corner (B) of the board, between which are the travelling mercury cups) by the insertion of plugs. Supposing the rheochord is interposed in a continuous current in place of a key, and to the binding screws the wires of the battery are attached, as well as the electrode wires : if the slide be close to the brass blocks and all the plugs in, the rheochord, offering no resistance to the current,

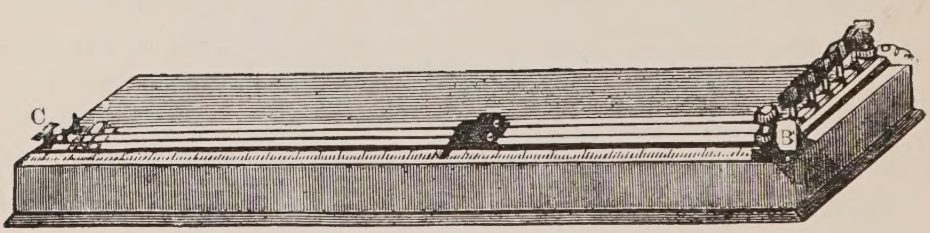

Fig. 32. Rheochord of Du Bois Reymond.

allows it to return to the battery, and none of it passes into the nerve. If the slide be pushed a short distance down the wires, the current meets with a certain amount of resistance, and so a fraction of the whole battery current will pass into the nerve. In like manner a greater and greater current passes into the nerve, if the slide be pushed farther away from the blocks, and if the plugs be removed one by one, as in this way the resistance offered to the passage of the battery current through the rheocord is more and more increased.

Effect of varying the Strength and Direction of the Current.—Take one or two Daniell's elements, and connect the wires from them with the rheochord binding screws, which should also be connected with a reverser or a commutator (Figs. 33, 34) ; if the latter, to the upper screws ; if the former, to the middle screws. The electrodes are connected to the lower screws of the commutator, and to the end screws of the

reverser. The handle of the commutator can be raised or lowered, and thus the direction of the current is changed.

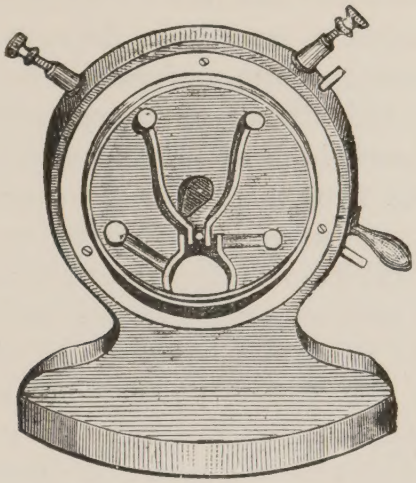

Fig. 33. Commutator.

When horizontal the current is cut off. In the reverser the current is changed by rotating the arch of wire, A B in the fig., to the right or left alternately.

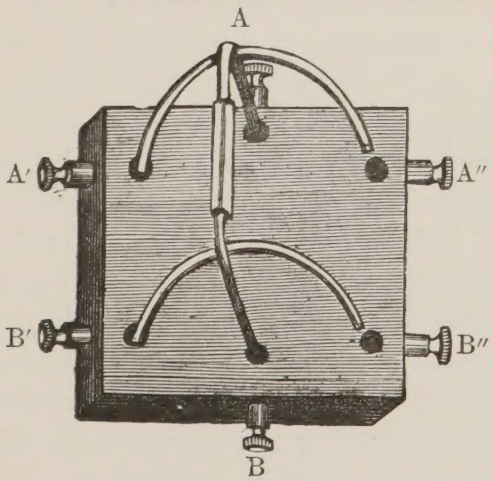

Fig. 34. Reverser. Brass wires (not shown in fig.) connect mercury cups corresponding to A′ and B″, and A″ and B′.

First of all put all the plugs in the rheochord, and push the mercury cups close to the blocks. No excitation occurs on raising or depressing the handle of the commutator. Gradually increase the strength of the current, and note the point at which

a contraction takes place as well as the direction, and whether it occurs at the make or break, or both. Make a table of the results and it will be found that the make contraction of the descending current is the first to occur, then the make of the ascending. Then, as the current becomes moderately strong with the make and break of both, and with a very strong current with the make of the descending and the break of the ascending.

The Rheoscopic Frog.—Prepare two nerve-muscle preparations, in the one case exposing the sciatic nerve throughout its length, and removing the skin from over the gastrocnemius only, but in the other case removing the leg with a long length of nerve. Insert the electrodes beneath the sciatic nerve of the first preparation ; place this on a glass plate, in order that it may be insulated. Place the sciatic nerve of the second preparation over the thigh muscles of the first, and excite the muscles of the first with a single induced current ; the muscles of the second will contract. Repeat with a series of shocks ; the second preparation will be thrown into tetanus, as well as the first. Show that this is not due to escape of the current by ligaturing the nerve. It is caused by the contraction of the muscles of the first producing a variation in their natural current (p. 236). This acts as a single stimulus to the nerve of the second, and so causes a contraction. Instead of passing a current through the nerve-muscle preparation, the nerve of the second may be dropped upon the muscle of the first preparation in such a way that one part of it falls upon the equator, whilst another part falls upon the muscle near its insertion into the tendon, or upon a transverse section of the muscle. The instant that the nerve falls upon these two points, the muscle of the second preparation will give a single contraction. The experiment may also be demonstrated by allowing the nerve of the limb to rest in a curve upon the exposed heart of the frog ; at each systole of the heart the muscles of the limb will contract.

Effect of Urari.—Carefully destroy the brain of a frog, without allowing the escape of any blood, inject a drop of a standard solution of urari, 0·1 per cent., into the posterior lymph-sac, after having dissected out the sciatic nerve of one leg, and ligature the limb tightly, in order to arrest the circulation in it,

excluding the nerve. Place aside under a glass shade for an hour, and test the excitability of both limbs. It will be found that the muscles of the ligatured limb will respond to stimuli applied both directly and also through the nerve, whereas the other limb will not respond to nerve stimulation, although it does so when the stimuli are applied directly to the muscle. This experiment shows that the poison has acted upon the nerve terminations, as it has affected neither nerve nor muscle.

2. **Mechanical stimulation.**—Prepare a nerve-muscle preparation : pinch or prick the nerve ; contraction will occur.

3. **Thermal stimulation.**—Touch the nerve or muscle of a nerve-muscle preparation with a hot needle ; a contraction will result.

4. **Chemical stimulation.**—Allow the nerve of the same or of a new nerve-muscle preparation to dip into a watch-glass full of strong saline solution ; flickering contractions which may pass into tetanus will result. A similar experiment may be performed with glycerine. Ammonia will stimulate muscle, but not nerve ; glycerine has the reverse effect.

GRAPHIC METHOD.

This consists in arranging the muscle-nerve preparation in such a way that, on contracting, the muscle raises or moves a lever, which lever is made to mark on a rapidly travelling surface. Various methods satisfy these requirements. In the first place, the muscle and nerve may be removed from the body, or may be retained *in situ*, and, again, the recording apparatus may be a revolving cylinder covered with blackened paper, or it may be a pendulum myograph or a spring myograph ; of all of which apparatus there are many varieties.

The Recording Cylinder (Fig. 35).—This apparatus consists of a cylinder or drum, which is arranged to move upon axes, revolving at definite and different rates, by means of a clockwork mechanism contained in a brass box, firmly resting upon supports. The velocities of the movement are (1) slow ; (2) medium ; (3) fast. The axes of 1 and 3 move in the same direction, viz., from right to left ; 2, on the other hand, moves

in the reverse direction. By means of a screw in the axis of the drum itself the drum may be raised or lowered at the will of the

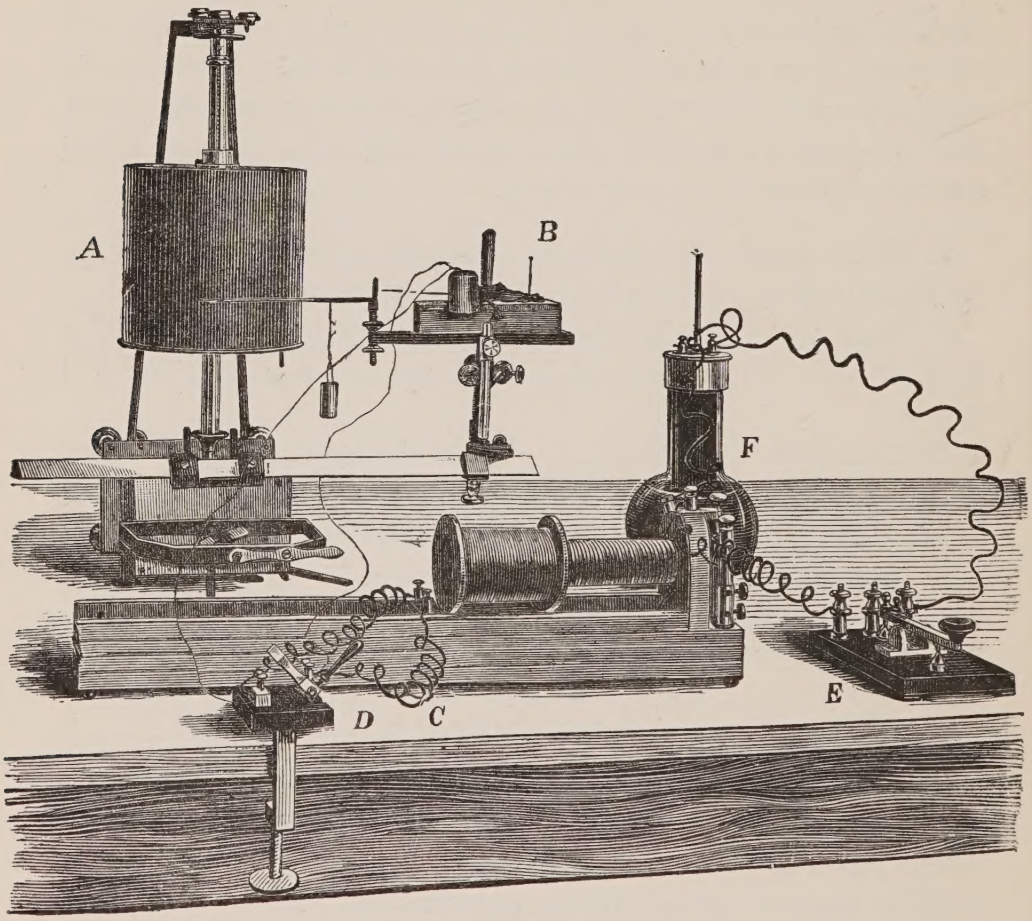

Fig. 35. Arrangement of Apparatus for recording with a revolving cylinder the Contractions of Muscle.

A. The cylinder or drum which is situated on the most anterior and most rapidly revolving axis. It is in connection with the brass box containing the clock-work. Two other axes are seen behind, and on either side of that bearing the drum.

B. Frog on a support; its muscle is connected by a thread with the Myograph: the whole being supported upon an upright springing from a triangular steel rod.

C. Du Bois Reymond's induction coil.

D. Du Bois Reymond's key.

E. Key in primary circuit.

F. Battery.

(*After Kirkes' Physiology.*)

operator. The apparatus works as well when placed in a horizontal as in a vertical position. In the front of the box is

arranged a fan in a metal frame, which regulates the clockwork movement. The movement may be stopped by means of a metal clip and handle, which are fixed so that when the handle is pressed down the clip catches a steel axis about which the fan revolves, and the clockwork is stopped. Underneath the case of the clockwork is a handle by which it is wound up. For the sake of convenience, in the front of the brass box of the clockwork are two screws, by which is fixed a metal frame, carrying a long, stout, triangular steel bar, which can be adjusted at different positions and lengths, by means of screws in the frame. On this triangular bar various metal uprights for carrying apparatus can be arranged. Also at one point of the lower edge of the drum is attached a metal catch for the purpose of opening a key placed in its way, called a *turn-over* or *trigger key* (Fig. 36), the use of which is to have the muscular contraction recorded exactly at the same place on the cylinder.

Having mastered the mechanism of the apparatus, the student will then arrange it for work. The drum must be evenly covered with glazed paper, which is generally kept ready cut in strips of the size of the drum. A strip is placed round the drum, care being taken that the join should not be near the metal catch mentioned above, as in that case the tracing of the lever will be over the join, and so be spoiled. Having firmly and evenly covered the drum, it must be blackened over the smoky flame of a small paraffin lamp or a spirit lamp, in the spirit of which camphor has been dissolved. For these operations the drum will have been removed from the clockwork ; it may now be replaced, the clock wound up, the metal bar firmly fixed, and everything arranged in readiness for the recording operation.

Method.—As above mentioned the muscle and nerve may remain *in situ*, or be removed from the body. First of all use the apparatus already described. This consists of a triangular piece of wood, covered to a certain extent with cork, and with an upright cylinder of the same material fixed at the side. In front is a lever arrangement, by which the movement of the muscle in contraction is communicated to the recording apparatus. On one side is fastened a small collar, which can slide up or down an upright fixed to the triangular

rod above described, and by means of a screw can be secured at any height which may be required.

In a nerve-muscle preparation, such as ii. (p. 216), remove the skin from the foreleg of the frog, and having cut through the tendon of the gastrocnemius at the *os calcis*, turn it up and cut off the remainder of the leg just below the knee-joint. Attach a strong ligature of silk or thread to the tendon, place the frog on the cork plate, firmly fix the limb by passing a long pin through the knee-joint, and then attach the ligature from the tendon to the metal at right angles to the marking lever, carefully noticing that the ligature is taut, and that the muscle is really pulling on the lever ; load the lever with a 10 or 20 grm. weight, and fix the myograph on the upright before spoken of, which slides along the triangular steel bar, so that the lever touches lightly the blackened surface of the recording cylinder, with the point writing the proper way. The apparatus is now arranged.

Moist Chamber.—This consists of a moveable platform, sliding up and down the upright of a stand not unlike a medium-sized retort stand, and capable of being fixed by means of a collar and screw. The platform is made of hard wood or of vulcanite, about $3\frac{1}{2}$ or 4 inches in diameter, and is furnished with two sets of binding screws for electrical purposes. These screws are continuous with wires which pass through the platform and project below, so that battery or other wires may be attached to them. In the front of the platform is an opening about one inch square ; upon the same upright slide, (1) an electrode holder, made very simply by fixing two copper wires in a small block of wood with the centre hollowed out, the wires being stretched across the hollow, and the end fixed in the wood ; they must be about one third of an inch apart, and are to be separated by a piece of cork. This wooden block is fixed upon a holder fitted with a collar and screw to move up and down the upright. (2) A brass rod, with the circular brass holder of a screw-clamp working easily in a collar at its end. The clamp holder can be fixed by a screw. A tall glass shade, large enough to cover the whole of the above, fits into a groove which runs round the platform a quarter of an inch from the edge. When in use, pieces of blot-

ting paper, wetted with water or saline solution, are inserted beneath the shade, to keep the contained air moist; hence the apparatus is called *a moist chamber*.

Under the platform of the moist chamber is attached a metal screw collar apparatus, similarly capable of movement up and down the upright of the stand, to which is attached a fine metal spring and lever of light wood, capable of movement up and down about a fulcrum near the collar. Having prepared a nerve-muscle preparation in a manner similar to that described above, but in addition having divided the sciatic, and turned it down over the muscle, clear the femur entirely of muscle, and divide at its lower third; then fix the femur in the clamp of the moist chamber, attach a ligature to the tendon (*tendo achillis*), and carry it through the opening of the stage to the lever below, which may be weighted in the same way as in the other apparatus, with ten to twenty grams; place the nerve on the electrodes, and bring the point of the lever to write on a cylinder as before. In the following experiments one or other of the above arrangements may be used.

a. Single Induced Currents.—Arrange the induction coil, battery and electrodes as described (p. 217), interposing the turn-over or kick-over key in the primary circuit. Cause the cylinder to revolve on the middle axis, with the key open. Before allowing the lever to touch the drum, find out the point at which the induction apparatus will give a sufficient stimulation; allow the drum to reach its proper rate of velocity; then, by means of a tangent screw, or some other delicate adjusting arrangement, make the lever touch the paper; let the catch pass the key, and then close it. In the next revolution the catch will open the primary circuit, and a contraction will occur. Mark the exact period of excitation by allowing the

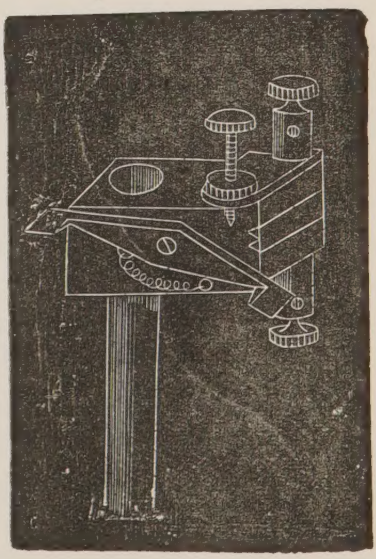

Fig. 36. Trigger or kick-over key.

cylinder to make nearly another revolution ; close the key, and gradually advance the cylinder, until the catch touches it, then slightly raise the lever, and allow it to mark the paper.

b. Faradization.—Arrange the key in the secondary circuit, and the apparatus as usual for this purpose (p. 219). When the hammer is properly working, allow the cylinder to revolve rapidly, and open the key. A curve of tetanus will be recorded.

c. Records of tetanus curves with spring, and also with metronome, and with vibrating reed, may be taken in a manner similar to the above, but use the medium speed axis.

d. Effects of Heat and Cold.—Arrange the cylinder on the second axis, allow the lever in connection with a nerve-muscle preparation to mark on the smoked paper, and set the clockwork in motion ; a straight abscissa line will be drawn. Interpose the kick-over key in the primary circuit, and by allowing the catch to open the key, record upon this line a single muscular contraction, and mark the latent period. Now cool down the muscle by filling a test-tube full of small pieces of ice, and bringing it for ten minutes into close proximity to the muscle. Then set the cylinder in motion ; and when everything is in readiness, remove the test-tube and close the key. On its next revolution the cylinder will open the key, and a muscular contraction will occur at the same point as before ; the curve will be found to be less sudden and more prolonged. By filling the test-tube with water of gradually increasing temperature, a series of curves may be taken on the same line, which will show that up to a certain point the curve will be more sudden and of shorter duration.

e. Effects of Poisons.—Inject to $\frac{1}{20}$ mgrm. of veratria into the posterior lymph-sac of a frog, and record the contraction of the gastrocnemius at various intervals after injection. It will be found that the full effect of this drug is to enormously lengthen the curve. Use medium speed. Other poisons may be tried in a similar manner.

f. Effect of Fatigue.—After using a muscle to demonstrate a single twitch, tetanus, and for other experiments, it will be found that the contraction will after a time alter, and that when

a single curve of a fatigued muscle is compared with that of a fresh one, it is found to be much more prolonged, and possibly less high, and that the latent period is longer.

g. Relation of the Contraction to the Load.—By loading a muscle with different weights, it will be found that with the same stimulus the contraction first of all increases as the load is increased from zero upwards by small increments. As the load continues to be increased, the increment diminishes, and finally gives place to a decrease. The initial increase of contraction is most prominent when its stimulus lies within a certain range of intensity.

h. Time Measurement.—This is done by the vibrating tuning fork, or by a reed made to vibrate a definite number of times in a second. The tuning fork of large size has on one prong a small style attached ; and after the prong has been smartly tapped by means of a mallet or similar instrument covered with felt, the style, if applied to the revolving cylinder, will mark the number of times the fork vibrates in a second. Another way is to place the tuning fork in a battery circuit, and allow its vibrations to be communicated to a small chronograph, which writes on the recording surface. This instrument consists of a small electro-magnet. Each time the iron is magnetised it draws down a piece of metal, arranged on a frame in such a way that it can move to or from the magnet ; at the other end of the frame is a small pedunculated hook, to which is fastened an elastic counterpoise. To the frame is fixed a style, capable of writing on a drum. Each time the rod vibrates, the current is made, the magnet draws down the piece of metal, and so the style makes a stroke on the smoked paper. At the break the elastic raises the style, and so on.

The Pendulum Myograph.—In this instrument, the clockwork movement, which is frequently unreliable, is replaced by the force of gravity. The recording plate is attached to a pendulum. On this principle several instruments, differing somewhat in detail, have been constructed. The pendulum with the recording plate is fixed by a catch which is capable of being moved certain distances along the arc through which the pen-

dulum swings. When the arc is lengthened, the velocity is altered. In its swing the pendulum knocks over, with a catch attached to the middle of its lower edge, a trigger key placed in the primary circuit, and by this means the muscle of a muscle-nerve preparation gives a contraction. The preparation is arranged in a manner similar to that described above (p. 229) for the other kind of recording apparatus; but the frog apparatus is fixed in a collar to the upright of a firm stand, capable of being raised or lowered by means of a screw. The upright is fitted with a circular movement worked by a tangent screw at its base. The stand, with the frog apparatus, is placed upon a firm table close to the myograph: this table can not only be raised and lowered, but is also capable of a circular horizontal movement.

Method.—Allow the pendulum to hang vertically. Arrange a nerve-muscle preparation on the table as above described. Cover the glass plate of the pendulum smoothly with glazed paper; having smoked it, replace the plate. Adjust the lever so that it barely touches the plate at its edge. Arrange a pair of electrodes under the nerve, interpose a key in the secondary current of an induction apparatus, and fix the trigger key (Fig. 36), at a convenient place in the battery circuit. Close the key in the secondary circuit (key 2), then raise the pendulum, and fix it in the catch to the right; see to the adjustment of the writing lever. Close the trigger key, then open the second key, and set free the pendulum. As it passes the trigger key, a stimulus will be sent into the nerve by opening the battery circuit, and a contraction will be recorded on the plate as it passes the lever. Underneath this tracing, a tracing of a vibrating tuning fork must be taken, and the latent period may be marked by returning the pendulum to its place, closing the second key, and then by carefully approaching the pendulum catch to the trigger key, which can be kept closed by the finger; by slightly raising the lever and allowing it to make a mark upon the paper, the exact point where the current entered the nerve is recorded. The effects of heat and cold, and of the action of poisons, can be demonstrated with this myograph, and also by taking two pairs of electrodes, and placing one a long

distance from, and the other near, the muscle, connecting them to the end screws of a reverser (Fig. 34), from which the cross wires have been removed, and alternately throwing the current into one or the other pair of electrodes ; the effect of having a long or a short piece of nerve stimulated may be shown by the difference in the length of the curve. By having two trigger keys arranged at small distances, two coils, batteries, and electrodes, the effect of two stimuli acting one after the other may be shown.

The Spring Myograph.—In this instrument the clockwork is replaced by the momentum imparted by a strong coiled spring. The recording surface is, as in the case of the pendulum myograph, a glass plate ; and in this instrument, as in the other, the glass plate in its course opens a trigger key, and a contraction similarly ensues, as the apparatus, induction coil, key, and electrodes are arranged in an almost exactly similar way.

Muscle Currents.—Arrange a Thomson's galvanometer in a dark chamber, and place the scale at about three feet east and west facing it. Light the paraffin lamp, and, after having set the mirror free by raising the screw adjustment at the top of the instrument, adjust the lamp so that the light falls well on it. By means of the magnet, adjust the mirror until it throws its light upon the zero of the scale. To the binding screws of the galvanometer attach the screws from the shunt, and for the sake of practice allow a weak current to pass from a Daniell ; by attaching wires from the battery to the shunt, with the plug in the hole marked $\frac{1}{999}$, the needle will be deflected, as indicated by the movement of the spot of light on the scale ; the light will probably move in the same direction as the current. Prepare two pairs of non-polarizable* electrodes, and connect them with the shunt ; the plugs being in the shunt, place the electrodes so that their plugs touch : on opening the shunt there will be no deflection. Now take a pithed frog, and having dissected out the sciatic nerve, fix the femur in a cork

* A non-polarizable electrode is made by half-filling a piece of glass tube with china clay moistened with saline solution and projecting as a plug from one end, and then filling up the tube with a saturated solution of zinc sulphate, into which dips an electrode of well-amalgamated zinc.

plate by means of a pin, and having attached a ligature to the tendo-achillis, tie it firmly to a hook, and stretch it slightly; remove the remainder of the frog from the gastrocnemius thus prepared; place the electrodes near, having inserted the plugs in the shunt so that one touches the middle, and the other one end; now open the plug $\frac{1}{9}$, the light will quickly pass off the scale; re-insert the plug, and remove another, so as to allow less of the muscle current to pass through the galvanometer, say $\frac{1}{1000}$; then note the amount and direction of the current, from the direction of the deflection and the degrees of the scale the light has passed over. Similarly the position of the electrodes may be altered, and the currents noted, and the general laws demonstrated. For the purpose of showing **the negative variation** of the muscle current when the muscle enters into contraction, arrange under the nerve a pair of electrodes connected with an induction coil; the rest of the apparatus being as before.

Electrotonus.—Prepare a nerve-muscle preparation, and remove it from the body, taking care that the sciatic nerve is uninjured, and very long. Place the preparation in a moist chamber, and arrange the nerve over two pairs of non-polarizable electrodes; connect one pair of electrodes with an induction coil, and arrange for single shocks; connect the other pair with a rheochord, the binding screws of which are also joined to the end screws of a reverser, to the middle screws of which the wires of one or more Daniell's elements are connected. Find out the exact minimal current which will cause a contraction, and then move the secondary coil a little farther away from the primary. On making or on breaking the primary current there will be no contraction; but if now a descending galvanic current, which is called the polarizing current, be sent into the nerve, an induction current weaker than the normal minimal will cause a contraction. This will also occur for some time after the galvanic current has been shut off. Similarly, it may be shown that if the current be ascending, an induced current, stronger than the minimal, will be required to produce a contraction; in like manner, the effects of a series of shocks may be shown, plus an ascending or descending continuous cur-

rent. So we see that the irritability of the nerve is increased during the passage of a constant current in a descending direction, and diminished if it be in a contrary direction. If the relative position of the electrodes of the induction and polarising current be reversed, an exactly opposite effect occurs in all particulars.

OTHER PROPERTIES OF MUSCLE.

Elasticity.—Prepare the gastrocnemius attached to the femur, clamp the femur, attach the gastrocnemius tendon to the lever of the moist chamber, and load the lever with a ten gram weight. Allow the lever to mark on a recording cylinder, then load with twenty to thirty grams, and so on. It will be found that the extensibility gradually diminishes for equal increments of weight. On removing the weights, the lever will return to the same point on the paper from which it started.

Reaction.—Remove from a pithed frog a gastrocnemius which has been perfectly freed from blood, cut it across with a sharp knife, and apply blue and red litmus paper to the ends. It will be found that there will be a bluish mark on the red litmus.

Transparency.—Take a flat muscle from a pithed frog (*e.g. the mylohyoid* or *sartorius*), and as soon as possible place it on a slide in saline solution. Examine with a quarter inch, and focus through the muscle some vessel underneath the fibres. It will be found quite clear, and so prove the transparency of living muscle. On entering into *rigor mortis* this property disappears.

CHAPTER III.

PHYSIOLOGY OF THE FROG'S HEART.

Anatomical considerations.—Open the abdominal cavity in a pithed frog by a longitudinal incision, avoiding the large veins in the middle line ; cut through the middle of the sternum and pin out the arms on either side ; pass a thick glass rod down the œsophagus, open the pericardium, and note the following points : the two auricles, the conus arteriosus on the right side, dividing into the two aortæ. Lift up the apex and the fine ligament or frænum connecting the dorsal aspect of the ventricle with the pericardium comes into view. Tie a fine ligature to the frænum, which can then be severed from the pericardium. When turned up, notice the auricles again and, behind, all the sinus venosus ; at the junction of the sinus with the right auricle, just above the auricular ventricular groove, is seen the white line. The sinus venosus is then formed by the confluence of the inferior vena cava, the two hepatic veins, and the two superior venæ cavæ. Note the sequence of the rhythmic contractions, of the sinus venosus, auricle, and ventricle.

Position of the ganglionic centres.—The vagi run in company with the superior venæ cavæ, and, on reaching the sinus venosus split into a plexus, which is beset with ganglion cells (*Remak's ganglia*). Some of the fibres are gathered together and pass on into the septum auricularum, where there are again many ganglion cells (*Bidder's ganglia*). These fibres pass down in the septum to the upper part of the ventricle, where there are secondary ganglionic masses, particularly on the dorsal aspect.

The inhibitory centre.—Turn up the ventricle and apply elect-

rodes to the white line above mentioned, stimulate with the interrupted current for a second or two, and notice that the heart stops in diastole, but soon goes on beating again.

Dissection to expose the vagus.— The easiest way to do this is to dissect away the integument over the scapula, cut through all the muscles attached to it, keeping close to the bone, divide the brachial nerves and vessels and so remove the scapula with the fore-limb attached. Close under the spot where the aorta of that side gives off the carotid and axillary vessels lies the cornu of the hyoid bone ; to this is inserted a thin strip of muscle which

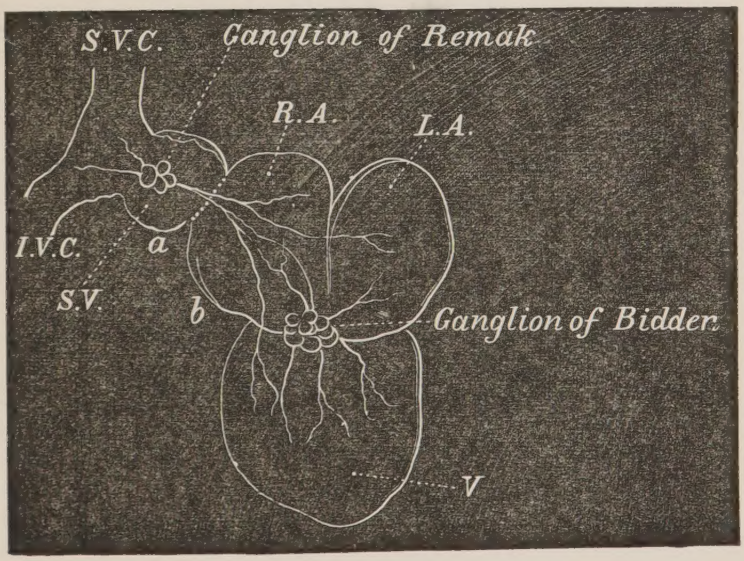

Fig. 37. Ganglia of Frog's Heart. *(After Carpenter's Physiology.)*

can be traced upwards and backwards to its origin on the petrous bone : this is the inferior petrohyoid. On this muscle lie three nerves ; the most anterior passes down for a short distance, and turns sharply forwards and upwards, towards the tongue (glosso-pharyngeal); the middle, a slender nerve, passes down the middle of the muscle to be distributed to the larynx (the laryngeal branch of vagus) ; on the posterior border of the muscle lies the vagus, which passes down over the root of the lung to the sinus venosus. The nerve generally lies under some pigmented veins and fascia.

Stimulate the vagus.—(*a*) With a very weak interrupted current, and with the arrangement known as Helmholtz modification. Notice that the beat is slow by lengthening the diastole. (*b*) With a strong current, the heart stops, after one beat, in diastole.

Intrinsic nervous mechanism.—(1) Excise the heart with a sharp pair of scissors, leaving behind the sinus venosus, place the excised auricles and ventricle into $\frac{1}{2}$ per cent. salt solution. Notice that the sinus goes on beating as before, but the excised

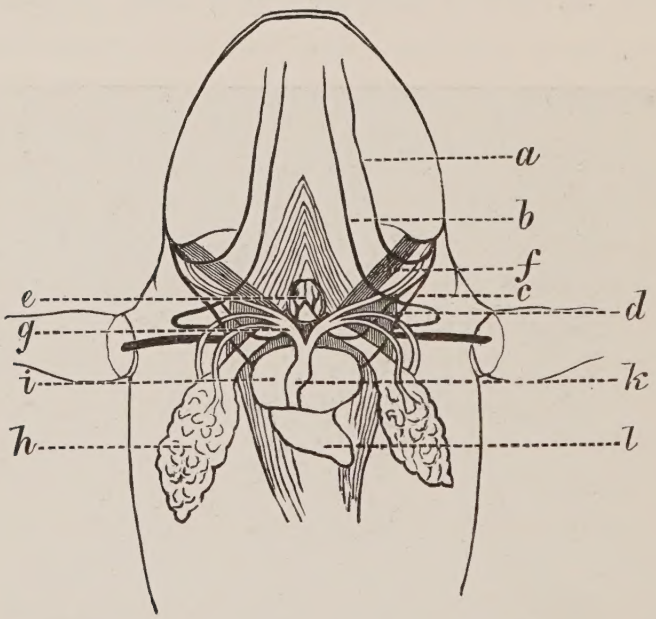

Fig. 38. Dissection showing position of Vagus in the Frog. (*a*) Glossopharyngeal nerve; (*b*) hypoglossal nerve; (*c*) vagus; (*d*) laryngeal nerve; (*e*) larynx; (*f*) petrohyoid muscle; (*g*) aorta; (*h*) lung; (*i*) auricle; (*k*) bulbus arteriosus; (*l*) ventricle.

heart stops for a time and then goes on again, especially if stimulated; the rhythm, however, is different from that of the sinus.

(2) Separate the auricles from the ventricle; both go on beating, but with independent rhythm.

(3) Separate the upper third of the ventricle from the lower two-thirds. The latter will no longer contract rhythmically, but will respond to a single stimulus with a single beat, the latent period being very long.

Stannius' experiment.—Pass a ligature between the auricle and sinus venosus below, and between the aortæ and venæ cavæ superiores above. Tie it tightly, so that the sinus is separated by the ligature from the rest of the heart. The heart stops in diastole, but the sinus beats naturally. On stimulating the flaccid heart single, and sometimes a succession of, beats may be obtained. If now the heart be separated by a clean incision along the line of the ligature, it will resume an independent rhythm to that of the sinus.

Action of certain poisons.—Muscarin or Pilocarpine.—Place the excised heart in neutral saline solution, with a trace of muscarin or pilocarpine solution. It will become quite motionless in diastole. *Atropin.*—Transfer the motionless heart to a ·2 per cent. solution of atropin in neutral saline solution. Notice the gradual return of the beat. Atropin is the antagonist of muscarin and pilocarpine.

Further action of Atropin.– Inject under the skin of the back a few drops of atropin solution, 1 per cent. After about ten minutes pith the frog and make a vagus preparation. Stimulate the vagus; no inhibition follows, even with the strongest currents. Stimulate the inhibitory centre, and again no stopping of the beat results. The vagus nerve-endings are paralysed. Stannius' experiment, however, succeeds as with the normal heart.

Frog-Heart and Rheoscopic Limb.—Prepare the hind leg of a vigorous frog, together with a long length of uninjured sciatic nerve, and in the same pithed frog expose the heart and open the pericardium. Arrange the frog on a glass plate, and also the limb on another, then allow the nerve to fall upon the ventricle. Each time the ventricle contracts, a contraction occurs in the limb.

Graphic Methods.—For this purpose a hollow cylindrical box, about three inches long and one inch in diameter, is fixed upon a metal support. The box is provided with two metal tubes, by means of which water at various temperatures may be passed through it by attaching to the metal tubes gutta-percha tubes, the one passing from a vessel fixed on a stand at some distance above the frog box, and the other similarly fixed somewhat

below. The lever is thus made : a glass rod, of the thickness usually employed as a stirrer, is taken, and with a blow-pipe flame it is softened sufficiently to allow of its being drawn out at the softened part to great fineness : the fine part is then broken at a point about five inches from the unaltered glass tube, which is now similarly drawn out the other side, leaving a knob of glass between the two thinner parts. On this side all the thinned part is removed, and the glass now remains with a thin arm about five inches long. A square piece of cork is now passed along the thin glass to the knob, and through this a fine needle is passed. The needle can be adjusted in bearings which are fixed to the edge of the box. A second piece of cork is passed along the lever arm, and is adjusted and cut so that its point, directed downwards, can rest upon the ventricle of the heart. After these corks have been put in place, the writing end of the lever may be made by allowing the extremity to be softened for a few seconds in the flame of a spirit lamp. The frog heart-box can be adjusted to the recording cylinder, and for the purpose of recording the contraction the cylinder should revolve slowly. (1) Having exposed the heart of a pithed frog, tie a ligature to its frænum, and, cutting through the vessels, lift it by the ligature to the heart-box ; having first allowed water at about 10° C. to pass through it, moisten the heart by placing a little serum on the top of the box by means of a capillary pipette. Adjust the lever so that the cork rests well upon the ventricle, and the writing lever marks on the recording drum record-tracings of the contractions at various temperatures, cooling the water down by means of ice to 1° C., and then raising it by increments to 20° C., and compare the tracings. (2) If, instead of a heart beating in the ordinary way, a *Stannius' preparation* (p. 241) be substituted, the heart may be stimulated by weak induction shocks, the apparatus being arranged so that the electrodes just touch the ventricle ; and with a key in the secondary circuit, and the kick-over key in the primary circuit, definite regular contractions may be compared, and the effects of temperature in altering the rapidity and the strength of the contraction seen, as well as the latent period recorded.

Endocardial Pressure.—A large frog (*Rana esculenta*) is taken, and the heart is exposed in the usual manner, the pericardium opened, the frænum ligatured, and the heart turned over by the ligature. A cut is made into the bulb, and by this means a double cannula is passed into the ventricle, a ligature is passed round the heart, and the cannula is tied in tightly. The vessels are then divided beyond the ligature, and the cannula, with the heart attached, is removed. To one stem of the cannula a tube is attached, communicating with a reservoir of a solution of dried blood in ·75 saline solution, and filtered, which is capable of being raised or lowered in temperature by being surrounded by a metal box which contains hot, cold, or iced water. Attached to the other end is a similar tube, which communicates by a T piece with a small mercury manometer, provided with a writing style, and also with a vessel into which the serum is received. The apparatus being arranged so that the movements of the mercury can be recorded by the float and the writing style on the slowly revolving drum, and after some serum has been allowed to pass freely through the ventricle, both tubes are clipped, the second one beyond the T piece, and the alterations in the pressure are recorded. The effects of fluids at various temperatures should similarly be recorded in the manner indicated above.

Roy's Tonometer. — By this apparatus the alterations in volume which a frog's heart undergoes during contraction are recorded by the following means : A small bell-jar, open above, but provided with a firmly fitting cork, in which is fixed a double cannula, is adjustable by a smoothly ground base upon a circular brass plate, about two to three inches in diameter. The junction is made complete by greasing the base with lard. In the plate, which is fixed to a stand adjustable on an upright, are two holes, one in the centre, a large one about one-third of an inch in diameter, to which is fixed below a brass grooved collar, about half an inch deep; the other hole is the opening into a pipe provided with a tap (stopcock). The opening provided with the collar is closed at the lower part with a membrane of animal tissue, which is loosely tied by means of a ligature around the groove at the lower edge of the collar. To this membrane a piece of cork is fastened by sealing-wax, from

which passes a wire, which can be attached to a lever, fixed on a stage below the apparatus. When using the apparatus, fix the bell-jar by means of cord, drop a little glycerin into the collar closed by membrane, and fill the jar with olive oil. Now prepare, in the way above described, the heart of a large frog, tie in the cannula, which is, as before mentioned, fixed in the cork; the tubes of the cannula communicating with the reservoir of serum on the one hand, and with a vessel to contain the serum after it has run through on the other. Pass the cannula with heart attached into the oil, and firmly secure the cork. Now open the tap, raise the membrane a little, and allow a few drops of the oil to pass out; shut the tap, and let go the membrane. By these means the lever will be found to be adjusted to a convenient elevation. Allow the lever to write on a moving drum, pass serum through at various temperatures, and compare the tracings. After a short time the heart will stop beating; but two wires are arranged, the one in the cannula, the other projecting from the plate in such a way that the heart can be moved against them by shifting the position of the bell-jar a little. The wires act as electrodes, and can be made to communicate with an induction apparatus, so that single induction shocks can be sent into the heart to produce contractions, and if need be, by means of the trigger key, at one definite point in the revolution of the recording cylinder.

CHAPTER IV.

NERVOUS SYSTEM.

Functions of the Medulla Oblongata in the Frog. — Hold the frog as if about to pith it (see p. 215) ; divide the skin, occipito-atlantoid membrane and medulla by a transverse cut with a sharp scalpel ; destroy the brain by thrusting a pointed piece of wood into it. The operation should be almost bloodless. The frog should be allowed to rest on its belly for a short time, to enable it to recover from the shock. Before long it will be found to have assumed a *nearly* normal attitude. It does not, however, make any spontaneous movement, provided that it is kept moist and at an equable temperature. If the flank be gently stroked, the muscles will twitch ; and if the stimulus be more violent, bilateral movements will occur. This is best seen in a frog which is freely suspended. If the skin about the anus be pinched, both legs will be simultaneously drawn up.

Functions of the Roots of the Spinal Nerves.—Divide the skin along the back of a frog, whose brain alone has been destroyed, as in the previous experiment. Separate the muscles of the back, so as to expose the arches of the vertebræ, which should be carefully cut away with a pair of blunt-pointed scissors. The roots of the nerves will then be seen within the spinal canal. Expose the roots of the eighth, ninth, and tenth nerves, taking the greatest care not to touch them, by completely removing the surrounding structures. The posterior roots will then be seen to be the larger and the more superficial ; they conceal the anterior roots. Select the largest of the roots now visible—it is that of the ninth nerve—and pass a fine silk ligature round it

without touching it more than is necessary. Tighten the ligature. At the same instant movements will be noticed in some part of the body of the frog. Cut the nerve between the ligature and the cord ; movement will again take place. Place the proximal portion of the divided nerve upon a pair of electrodes in connection with a Du Bois Reymond's induction apparatus ; decided movements will occur on the passage of a current, whilst no such movements are seen when the distal extremity of the nerve is stimulated in the same manner.

Cut away the posterior root, and repeat the preceding experiments with the anterior root ; movements will occur as soon as the root is touched, as well as when the ligature is tightened, and when the nerve is divided. Tetanus ensues upon stimulation of the distal portion of the nerve ; but the passage of an electric current through the central end produces no result.

Functions of the Spinal Cord.—In a preparation in which the brain has been destroyed, and the cord divided below the medulla, (*a*) the reflex function of the cord may be shown by irritating the surface of the skin by means of small pieces of filter paper dipped in acid, and placed in various situations ; contraction of certain muscles usually follows for the purpose of getting rid of the irritation. The groups of muscles are as a rule regularly brought into action when particular parts are irritated. (*b*) If the irritation occur in one leg, and that leg be prevented from moving, the other leg will act ; but after a time general contraction may occur. (*c*) If the irritation be very great, or if the frog be under the influence of strychnia, general convulsions may occur.

APPENDIX.

ON THE PRESERVATION OF NORMAL AND PATHOLOGICAL MUSEUM PREPARATIONS.

The only satisfactory method of making permanent preparations of entire organs or tissues is to obtain the specimen whilst it is quite fresh. When this can be done it is placed in a zinc or earthenware basin, and a small stream of fresh water is allowed to flow upon it for twenty-four hours. At the end of this time it is deprived of the blood which it contains by gently squeezing along the course of the main vessels. It is then dissected in such a manner as to show the especial features for which it is preserved. It will be found that the dissection is more readily and cleanly performed at this stage than after the preparation has been placed in spirit. It is then put back into the basin and the water is allowed to flow over it until the greater part of its colouring matter has been washed away. In cold weather this is readily effected, but in summer it must be carefully watched to prevent decomposition setting in. The amount of water which flows over the specimen is of less importance than its constant renewal.

As soon as the washing is complete, which will usually be in forty-eight to seventy-two hours after its commencement, the preparation should be suspended by means of silken threads in the glass jar in which it will eventually be preserved. A mixture consisting of half methylated spirit and half water is then poured over it. The bottle should afterwards be conspicuously labelled and set aside, the spirit being changed as often as it becomes discoloured. In dealing with large numbers of specimens it will be found convenient to place in each jar an unground microscopic slide or other slip of glass, upon which a reference to the specimen has been made by means of a writing diamond, details being at the same time entered in a book kept for the purpose.

As soon as the spirit in which the specimen is immersed remains clear, the final dissection should be made under weak spirit, all loose pieces of connective tissue being removed with a pair of curved scissors, the muscles being carefully cleaned, and the various points of interest being demonstrated to their greatest advantage. The preparation is then suspended in a mixture of proof spirit and water in the proportion of one part of spirit to two of water. Proof spirit is employed as the permanant preservative agent because it does not become cloudy with sudden atmospheric changes, as is so frequently the case with methylated alcohol. The glass jar must be provided with a ground rim, upon which a glass cover of moderate thickness fits flush. The cover is luted to the bottle by a cement prepared according to the following formula :

Melt together, in an iron or earthenware vessel, one pound of gutta-percha and one pound of asphalt, and when they are thoroughly mingled pour them on a slate or stone slab which has previously been moistened with water. Roll the mass into cylinders of convenient length and thickness, and use the sticks in the same way as sealing-wax. The glass cover should be gently warmed before it is pressed upon the hot cement which has been spread along the rim of the glass jar. A weight, varying from four to twelve pounds according to the size of the jar, is placed upon the freshly-cemented top for a period of forty-eight hours, after which the superfluous cement is scraped away and a neatly applied ring of Brunswick black is painted round the edge and top of the cover in such a way as to conceal the line of junction.

The method here detailed is not applicable to every specimen. The hollow viscera, such as the uterus, bladder, etc., after they have been washed for twenty-four hours, and after the removal of redundant tissue, should be stuffed with tow, cotton-wool, or horse-hair, the aperture being subsequently sewn up. They should then be suspended for seventy-two hours in 64 per cent. methylated spirit, after which the stuffing should be removed, the preparation being placed in the ordinary mixture of methylated spirit and water, until they are ready for mounting. These preparations are put into strong spirit to cause them to set in the form given to them by the stuffing. Structures which deteriorate in water, such as the brain and other nervous tissues, and those in which it is desired to preserve, as far as possible, the original colour, should be placed at once in strong spirit.

Large anatomical and physiological preparations may be embedded in plaster of Paris, in white earthenware basins with ground rims, the basin being subsequently filled with diluted proof spirit, and the cover sealed down with a cement made by mixing together litharge and gold size to form a paste. As in the case of the glass jar, a weight must be placed

upon the cover to keep it firmly adherent to the basin until the cement begins to dry, which will not usually take place much under a week.

Specimens showing colloid degeneration, tubercular synovitis, villous tumours, etc., may be preserved in a mixture of equal parts of glycerine and distilled water, to which has been added a trace of corrosive sublimate (1 part in 1500) or of pure carbolic acid.

In a few cases where it is necessary to preserve as far as possible the colour and translucency of the preparation, as in voluntary muscle infested with trichinæ, Wickersheimer's fluid may be employed. This fluid can be obtained from the importers, Messrs. W. Schacht and Co., 26, Finsbury Pavement, E.C., at a price of 2s. 6d. per quart bottle, or it may be manufactured according to the accompanying formula :

Alum	100 grammes.
Caustic potash	60 ,,
Chloride of soda... ...	25 ,,
Nitrate of potash ...	12 ,,
Arsenious acid	10 ,,

Dissolve the ingredients in three litres of boiling water. Cool. Filter, and afterwards add to each litre of fluid 400 c.c. of glycerine and 100 c.c. of proof spirit.

The fresh preparation should be entirely immersed in the fluid for ten days, and it should then be suspended in a glass jar with a small quantity of the fluid beneath it. The jar should be sealed in the usual manner. Neither the glycerine process nor Wickersheimer's fluid, however, have been found to yield very satisfactory results, and for valuable specimens it is better to adopt the alcohol method.

Small specimens, such as ovaries, sections of human eyes, etc., may be conveniently preserved and shown, by means of glycerine jelly, in the following manner :

After clearing away all redundant tissue, the specimen should be placed in Müller's fluid for a month. If its contents are suspected to be fluid, or semi-fluid, it should be frozen by placing it in a mixture of powdered ice and salt, and whilst it is hard it should be divided in the required direction with an ordinary sharp table-knife. The colouring matter of the Müller's fluid must then be dissolved out by placing the specimen in a solution of chloral hydrate (40 grains to the ounce), the chloral being changed as often as it becomes discoloured. The preparation is then placed for two or three days in a mixture consisting of one part of glycerine and three parts of water, from which it is transferred, for forty-eight hours, to a stronger mixture of equal parts of glycerine and water. It is then ready to mount in glycerine jelly, which is made

thus: One part of Coignet's (Paris) gold label gelatin* is soaked in six parts of distilled water until it is thoroughly swollen. It is then heated, and six parts of glycerine, to which a trace of pure carbolic acid has been previously added, is mixed with it, and the whole is filtered whilst it is hot through white filtering paper or cotton-wool—the heat being maintained during the filtration by means of a funnel with a hot-water jacket. The resulting jelly is preserved for use in a stoppered bottle.

To mount in glycerine jelly, small glass capsules similar to those employed in histological work are required, but they should be made of thicker and more brilliant glass. The jelly is liquefied by placing the bottle which contains it in hot water, and it is then poured over the preparation, which has been previously placed in the capsule. In the case of sections of the eye, care must be taken not to pour the jelly over the cut edge, lest the retina be detached. All bubbles should be carefully removed, and whilst the jelly is setting the specimen should, if necessary, be kept below the surface by gently pressing upon it with a needle fixed in a handle. When the jelly is quite firm its surface should be covered with an even coating of zinc paint, over which again a layer of shellac may be spread.

The glass jars should be round and of clear white glass free from bubbles. Large oval bottles frequently crack spontaneously, owing, so far as can be ascertained, to the extreme difficulty which is experienced in getting them properly annealed. The square bottles made in Holland, although very much cheaper and useful as stock bottles, are not of sufficiently good glass to be of much service in a well-kept museum. The covers of the basins, as well as of the large glass jars, should be provided with a hole plugged with a well-ground glass stopper—as, apart from the convenience of filling them without disturbing the cement, it will serve to prevent the covers cracking across as a result of variations in the bulk of the spirit due to alterations in the atmospheric conditions.

THE ORGAN OF JACOBSON.

Preparation.—By placing the anterior portion of the head of a young guinea-pig in a $\frac{1}{4}$ per cent. solution of osmic acid for twenty-four hours, and subsequently in absolute alcohol for 48 hours. A trace of hydrochloric acid is added to decalcify the bone, and the sections are made in a plane parallel to the tip of the nose.

* Price 2s. 10d. per pound. The ordinary gelatins sold in packets in this country, even Nelson's No. 1 gelatin, used for photographic purposes, will not give a transparent jelly.

Structure.—The organ of Jacobson is bilateral, and consists of a tube lying in the lower or osseous part of the septum narium. It is flattened when seen in transverse section, so as to appear kidney-shaped, and is surrounded by a special investment of hyaline cartilage which is frequently incomplete. This cartilage is known as Jacobson's cartilage. The epithelium lining the tube is similar to that covering the mucous membrane of the nasal cavity, but the epithelium lining the inner or mesial side of the canal is thicker than that on the outer side. The thicker epithelium is called the sensory epithelium. Beneath the epithelium is a fibrous layer, and still deeper are glands and a tissue consisting of a cavernous system of bloodvessels. Numerous branches of the olfactory nerve supply the organ, which also contains a few unstriated muscle fibres. Nothing is known of its function except that it is accessory to the sense of smell.

PREPARATION OF UREA.

Concentrate urine to a fourth of its bulk, and add a cold saturated solution of oxalic acid. This throws down crystals of oxalate ; remove them and boil with some chalk, and a little water. Oxalate of lime is precipitated, and can be filtered off together with the excess of chalk. Carbonic acid is given off, and urea remains in solution, and may be crystallized out by concentrating (Berzelius).

Or mix urine with animal charcoal and evaporate gently to dryness. Extract with alcohol and concentrate till a drop will crystallize, then set aside to cool.

BACTERIA STAINING.

It may be useful, and not out of place, to devote a few lines to the subject of bacteria staining.

(1) *Staining cover-glass films of dried material*, e.g., sputum, pus, blood, or masses of micro-organisms from a cultivation. The material should be spread out in the thinnest possible film upon a cover-glass, dried in the flame of a spirit-lamp to coagulate all the albumen, and placed with the film downwards upon some drops of the staining fluid in a watch-glass for five minutes. It should then be washed quickly in dilute spirit, then in distilled water, dried in the flame of a spirit-lamp, and mounted in Canada balsam. Sometimes the washing has to be done in acid solutions instead of in water. This is the case with the bacilli of tubercle. In that case, too, double-staining should be used, as will be described below.

(2) *Staining sections of tissues* should be done in much the same way, but after staining in one dye it is usual to pass them through a second to double-stain. In this way the bacteria are stained one colour, whilst

the tissue itself is another. The combinations are very various. After double-staining, the sections are passed rapidly through the dehydrating and clearing solutions, and mounted in Canada balsam.

For single stains the following solutions are most useful :

i. An aqueous solution of Spiller's purple, to which a few drops of spirit have been added.

ii. An aqueous solution of methylen blue.

For double stains, it is only necessary to give the following combinations out of very many :

i. Stain No. 1. *Fuchsine*, made thus :

Anilin oil, 5 to 25 c.c.
Distilled water, 100 c.c.
Shake well together and filter.

To 100 c.c. of anilin water thus prepared add 11 c.c. of alcoholic solution of fuchsine.

Stain the sections for at least thirty minutes, slightly warming the solution. Wash in nitric acid, 1 in 3, then in water, and transfer to

Stain No. 2. *Methylen blue*, or *Vesuvin*, the former a saturated watery solution, the latter a 1 to 2 per cent. solution for a few minutes. Wash in alcohol, clear in clove oil or oil of cedar, and mount in Canada balsam.

ii. Weigert's 1st Stain :

2 per cent. watery solution of gentian violet ... 12 c.c.
Saturated watery solution of anilin ... ... 100 c.c.
Mix.

2nd Stain :

Bismarck brown... ... ... 1 grm.
Sp. Vini rect. (sp. gr. ·830) ... 10 c.c.
Distilled water ... ... 100 c.c.
Fifteen minutes in this.

(Recommended by Klein for tubercle, who, however, states that the colour fades.)

iii. Ehrlich-Weigert's formula for sections :

Saturated watery solution of anilin ... 100 cc.
Saturated alcoholic solution of fuchsin, or methyl violet, etc. ... ... 11 cc.

After staining in Ehrlich-Weigert's fuchsin, wash in distilled water, immerse it in alcohol for a moment, and then place in the following solution :

Distilled water ... ... 100 cc.
Saturated alcoholic solution of methylen blue ... ... 20 cc.

Formic acid (pure)	}	... ...	10 min.
(Or glacial acetic acid)	}		

Leave the sections in this solution for one to two hours, and then treat in the ordinary manner with water, alcohol, and oil of cloves.

iv. For *anthrax*, a combination of aqueous *methylen blue* (saturated solution) *and eosin* (1 per cent. in alcohol) is used. The eosin solution is added drop by drop to a little of the methylen blue in a watch-glass, as long as the precipitate which forms is redissolved. Stain for 1 to 3 minutes, wash in spirit, fix in commercial benzine, clear in a mixture of benzine and oil of cloves, and mount in Canada balsam.

For staining tubercle bacilli in tissues, we strongly recommend the following method :

First of all half fill an ordinary-sized test-tube with a solution of fuchsin in anilin water, as in the above method iii., but with somewhat more fuchsin solution, say 15 per cent. of the alcoholic solution, adding a few drops of absolute alcohol to keep it in solution ; warm the solution to about 45° C., filter into a porcelain dish, and keep the solution at about the same temperature with a spirit lamp ; place the sections in this solution and keep them in it for half an hour (or cover-glass specimens ten minutes) then transfer into the washing fluid thus made :

Of a 10 per cent. solution of nitric acid	...	...	1 part,
Methylated spirit	...	...	1 part,
Distilled water	...	...	1 part,

and wash thoroughly for several minutes.

Then transfer to a filtered solution of methylen blue (saturated) for 5-10 minutes. Afterwards wash in a saturated solution of sodium carbonate for 15 minutes, then in water. Next take the specimens out one by one on a section lifter, dehydrate quickly with absolute alcohol, and place upon a glass slide. Then allow a few drops of the turpentine and creasote mixture to fall upon the section, inclining the slide so that the fluid quickly runs from it as soon as the latter appears transparent. Remove the clearing fluid with filter paper and mount in Canada balsam.

Although a long process, by its use the crystals so often present in the nitric acid washed specimens are avoided, and the methylen blue forms an excellent background for the red bacilli.

FORMULÆ.

The following formulæ may prove useful :

Cohn's Normal Fluid.

·1 grm. Potassium phosphate.
·1 ,, Magnesium sulphate (crystallised).
·01 ,, Calcium triple phosphate.
20 c.c. Distilled water.

And also the above with ·2 grm. ammonium tartrate added, for 'cultivation' purposes.

Pasteur's Fluid :

Potassium phosphate	20	parts.
Calcium ,, ...	2	,,
Magnesium sulphate	2	,,
Ammonium tartrate	100	,,
Cane sugar	1,500	,,
Water	8,476	,,
	10,000	

Cane sugar to be omitted when Pasteur's fluid without sugar is required (Huxley and Martin).

Mayer's Solution (with Pepsin) :

15 per cent. solution sugar-candy ...	20	c.c.
Dihydropotassic phosphate	·1	grm.
Calcic phosphate	·1	,,
Magnesic sulphate	·1	,,
Pepsin	·23	,,

(Huxley and Martin.)

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